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A CONTRIBUTION TO THE KNOWLEDGE OF PYÆMIC
FORMS OF INFECTION IN SHEEP.

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THIS paper gives an account of some investigations made by the writer concerning a disease in sheep, a form of pyæmia that clinically suggests pseudo-tuberculosis in sheep, or what is called caseous lymphadenitis in England and suppuration caséuse in France. The affection is characterised by abscesses of various sizes, in the majority of cases concentrated in the head, especially the cheeks and their lymph glands, which localisation however, is not considered to be typical for *Preisz-Guinard's* sheep disease. The affection is quite distinct from pseudo-tuberculosis, pyobacillosis, and "Mal de Lure" as described by *Carré* in France, inasmuch as no Gram-positive rods are present in the pus. It has been possible to show a specific organism as the cause of the disease, and this is in many respects quite different from the pus-producers in sheep described hitherto.

The first to describe the disease and its real cause was Prof. M. Christiansen. As Research Director at Prof. C. O. Jensen's Institute at Copenhagen, he gave an account in 1917, in "*Maanedskr. f. Dyrlaeger*," of some observations made in 1916 on three sheep from one and the same flock in Denmark. He could not find that the disease and its organism had been previously described in the literature and therefore suggested the name *Bacterium purifaciens* for the bacillus in question. This investigation has, oddly enough, been almost entirely ignored in the hand-books on sheep diseases.

The first case of this disease to come under my notice in Sweden was in 1918, and since then I have investigated similar outbreaks in three different estates. I have also been informed that the disease was discovered at the Royal Veterinary Bacteriological Institute at Stockholm in 1927. It appears to me very probable that it will also be met with in other countries if only the attention of investigators is

directed to it. One is inclined to the belief that it has hitherto been confused with tuberculosis and pseudo-tuberculosis or caseous lymphadenitis. In *Marek's* "Lehrbuch d. klinischen Diagnostik der inneren Krankheiten d. Haustiere" there is an illustration, which is also reproduced in *Oppermann's* text-book of sheep diseases, representing pseudo-tuberculosis nodules in the skin of the head, but which very strongly suggests the morbid picture I shall describe in the following pages.

Case 1. On January 30th, 1920, the head of a sheep was sent to the Laboratory by Prof. B. Hedelin. The animal, which belonged to one of the largest sheep-farms in Sweden, had been ill for four weeks. The illness began with a swelling of the lower lip and the whole of the lower jaw. The animal had great difficulty in grazing and chewing the food. It had to be fed with chopped beet and concentrated food in order to keep it alive. But even this was of no avail in the long run, and four or five days before its death the animal had been unable to eat at all. The flock to which this animal belonged was kept grazing almost all the year round in some pasture land along the shores of The Sound.

When killed the animal was, according to information, in very good condition. The lungs and other internal organs appeared to be quite normal. The head showed a distinct swelling on the under side of the lower jaw and above the parotid region on both sides. In the nostrils there was slimy, greyish exudation. The whole of the lower lip was swollen to almost twice its normal size. The skin over the lower lip was ulcerous and covered with a scab that was easily removed. An incision into the lower lip showed the tissue to be thoroughly moistened by a reddish fluid but nevertheless very firm and fibrous. Moreover, abscesses appeared everywhere, varying in size up to that of a hazel-nut. The mucous membrane showed no changes. Similar abscesses were found in the subcutaneous connective tissue and between the muscles from the lower lip right down to the throat. They were spread along the submandibular region and in the cheek itself. They were most numerous in the right cheek, where they could be followed right in under *m. massiter*. Some ten of the abscesses had already opened outwards, with fistulæ which pierced the skin at various places above the ramus of the lower jaw. Altogether there were about fifty abscesses. The majority were the size of peas, but at least fifteen of them attained the size of a common hazel nut. The abscesses were not everywhere round but sometimes presented irregular processes from the wall. No well-marked capsule was present. On the other hand, there was in the majority of cases a sharply defined, narrow, hæmorrhagic zone around the separate suppurative foci. The pus was very viscid and was like greenish-yellow, inodorous, plastic dough, which could only be broken up with difficulty with a knife or a pair of forceps. Both subparotideal lymphatic glands were enlarged, succulent, and red, and contained each two to three pea-sized abscesses. This was also the case with the submaxillary and retropharyngeal lymph glands. The mucous membrane of the mouth showed no morbid changes. On the inside of the right cheek, however, there were a few round necroses, which were found to be located directly over small abscesses in the wall. Further, a thin, catarrhal, purulent, diffuse coating was observed on the mucous membrane of the pharynx. The tongue and teeth exhibited nothing worth noting. The nasal cavity, the eyes, the ears, and the brain were quite normal. No tubercle bacilli could be shown, *nor any Gram-positive bacteria*. Guinea-pigs inoculated subcutaneously with emulsions of abscess pus gave negative results after six months' observation.

The head in question arrived at the Laboratory about ten hours after the death of the sheep and, as it was a cold winter day, the probability of a post-mortem entrance of bacteria into the different abscesses could be excluded. By spreading a clump of pus the size of a pin-head on serum agar plates about sixty colonies were produced on the first plate and sixteen on the second. No colonies were obtained on the third plate. Smear preparations were also made from five other abscesses, one of which was in the left submaxillary lymphatic glands and two in the subcutis. Only colonies of one kind were obtained. It should be pointed out, however, that only four to five colonies were obtained in the first suspensions from the subparotoid and retropharyngeal lymph glands while growths of a greater number of colonies were got from other spots. The number of bacteria in the material was thus obviously small. The isolated bacillus proved to be identical with that isolated by Christiansen.

Case 2. On April 15th, 1921, Prof. Hedelin sent to the Laboratory, from the same farm as the preceding, the head, lungs, heart, liver, kidneys, spleen, and udder of a full-grown sheep that had been in good condition and was apparently healthy. The udder was damaged and the sheep was therefore slaughtered, when it was observed that there was a great number of ulcers in the various organs, which were therefore sent in for examination.

The head showed two abscesses as large as walnuts situated in the right cheek. In the palatine curtain there was another of the same size. Both the retropharyngeal and the submaxillary lymphatic glands were large and ulcerous on the right side. In the kidney there was a bean-sized abscess. There were five abscesses in the liver and four in the lung, one of the latter being as large as a hen's egg in circumference. Both halves of the udder were simply one mass of abscesses. These contained a very viscid, thick, green pus without a smell. The abscess walls were fibrous and fairly thick. Microscopically no tubercle bacilli and no Gram-positive bacteria were met with in the pus, but only a few Gram-negative rods. Gram-negative rods of the same type as in the preceding case were obtained culturally.

Two guinea-pigs were inoculated subcutaneously with an emulsion of the pus but showed no sign of tuberculosis six weeks after the inoculation.

Case 3. January 30th, 1926. On a farm having pasturage on the south coast of Scania, several sheep had had swellings and ulcers in the head and a live sheep was therefore sent in for examination. The flock consisted of seventy-one animals. Two had died and four were ill.

The animal was in poor condition but fed normally and had no fever. On the outside of the right lower jaw there were several scars without any wool. On the right side there was a distended abscess about the size of a hen's egg (*see* Fig. 1). On the under side of the lower jaw, at its angle, another ulcer could be felt deep down. On incision a quantity of viscid pus welled out. When the abscesses had been opened the skin healed up again, leaving only a couple of hairless scars. Post-mortem examination a few months later showed no foci in the lungs or in the lymphatic glands. No Gram-positive or acid-fast bacteria could be shown in the pus. Each of two guinea-pigs and two rabbits was inoculated intraperitoneally with an emulsion of a pea-sized clump of pus. After six months' observation the animals showed no symptoms of infection. Films on agar plates from a clump of pus the size of half a pea gave a growth of some eighty colonies on the first plate. All were of the same species and could be identified as *Bact. purifaciens*.

Case 4. On April 15th, 1926 another living full-grown sheep (see Fig. 2) was received from the same source as the preceding. For the past month it had shown swelling in the region of the neck and had commenced to lose weight.



FIG. 1.
Subcutaneous abscess caused
by *Bact. purifaciens*.
Case III. Original.



FIG. 2.
Abscesses in throat and
subparotideal lymph gland.
Bact. purifaciens.
Case IV. Original.

On arrival the animal was observed to have fluctuating, thin-walled, cold abscesses on both sides of the parotoid region. When incised with the knife these abscesses emitted a viscid, plastic mass of pus. The sheep got worse and worse and died with symptoms of suffocation on the 1st May. On section the submaxillary and subparotideal lymphatic glands were found to be enlarged and closely beset with abscesses. Numerous abscesses, varying from the size of a pea to that of a bean, were found in the cheek itself right up to the corner of the mouth. In the tonsils, the soft palate, the wall of the pharynx, and the retropharyngeal lymphatic glands were also situated a large number of abscesses. Owing to this mass of small and large suppurative foci the pharynx was quite constricted. The right lung showed two abscesses and the left lung three, each the size of a hazel nut. All the abscesses in the lungs were surrounded by a capsule about 2 mm. in thickness. The contents of the abscesses were green in colour, viscid and without smell. No Gram-positive bacteria or tubercle bacilli could be shown in the pus. Subcutaneous inoculation of guinea-pigs for the presence of tuberculosis gave negative results. Spreading on agar plates of a normal loop of pus from each of three different abscesses gave in the first plate 30, 44, and 100 colonies respectively. They were all of the same kind and identical with *B. purifaciens*.

Case 5. On October 24th, 1918, W. Borg, District Veterinary Surgeon, sent in the pluck together with the diaphragm, the liver, and one kidney of a sheep which had been examined when slaughtered at Sjöbo. The head was not received. According to information supplied this animal was full-grown and in a very fat condition and had been purchased from a sheep-owner who had no pasturage along the coast-line. It had not shown any signs of illness.



FIG. 3.

Metastatic abscesses in the lung and related lymph glands. *Bact. purificiens*.
(Photo by Christiansen.)

The general condition was good. The lungs and their lymphatic glands showed multiple abscesses. The pleura was smooth and glossy. The peritoneum was granular and had lesions like those of perlsucht in cattle. They were, however, not well-defined and pedunculate nodules like those most frequently met with in cattle in tuberculosis, but like a diffuse tumour, rather thick, 0.3-0.8 cm., and with an uneven, verrucose surface. The entire diaphragm was cemented to the liver so that the liver capsule came along with the diaphragm when the latter organ was dissected. In the liver there were five abscesses, situated partly deep down, partly arching above the free surface. One of the kidneys was converted into a single large abscess. The other exhibited a pea-sized abscess on the surface.

In the joints nothing worthy of note was found. The musculature and the intermuscular lymph glands were normal.

In the lungs, which resembled Christiansen's picture (see Fig. 3), there were some twenty abscesses, varying from the size of a pea to that of a walnut. They were surrounded by a capsule of connective tissue from 1 to 2 mm. in thickness. The surrounding lung tissue showed some compression but was otherwise normal. The abscesses contained viscid, semi-liquid, green pus. It was nowhere caseous or calcareous. The lymphatic glands of both the liver and the lungs were much enlarged and changed by abscess formation. The

left bronchial lymphatic gland was especially large, like a hen's egg, and showed a walnut-sized abscess that extended into and communicated with a similar abscess in the section nearest the left lung.

Microscopic examination of smear preparations from the different abscesses and from the tumour on the peritoneum showed no tubercle bacilli or any other bacteria. Cultures were prepared with half a normal loop of pus on various culture media, and the first suspension yielded in most cases a growth of fifteen to twenty colonies. They were cultivated partly from the abscess in the bronchial lymphatic gland, partly from one of the abscesses in the liver. The colonies were all of the same kind and consisted of Gram-negative rods. In their cultural properties they agreed in all essentials with Christiansen's *B. purifaciens*.

Histological examination of the diffuse peritoneal growth showed a rather fibrous granulation tissue, richly beset with pus cells. No giant cells of Langhan's type were found. Here and there were noticed an abundance of minute abscesses, in the centre of which clusters of oval rods were sometimes encountered. Guinea-pigs inoculated with pus gave negative results. There could therefore be no question of tuberculosis.

Case 6. March 28th, 1922. In March the head of a sheep was received from a large sheep farm on an island in Kattegat off the coast of Holland. This animal had exhibited for many months swellings in the mouth and cheek and owing to its inability to feed was slaughtered (see Fig. 4). No other sheep in the flock had shown any signs of infection.



FIG. 4.

Diffuse pustulous dermatitis and lymphadenitis caused by *Bact. purifaciens*. Case VI. Original.

The entire mouth was considerably swollen. The skin was bare of wool and showed numerous punctiform, pea-sized, rounded protuberances. When pressed these protuberances emitted a large quantity of viscid, green pus. The whole of the left cheek was bare and was also beset with abscesses. The right cheek was also in the same condition but had besides, just behind the corner of the mouth, a square ulcer 3 cm. in length, and having an uneven purulent bottom. Inspection of a cut into the skin of the lip showed an innumerable mass of subcutaneous abscesses. The underlying tissue was extremely infiltrated. The abscesses were encountered right back to the parotid region. Here they were in many cases as large as hazel-nuts. The

submaxillary lymph glands were large, succulent, and harder than usual. Both retropharyngeal lymph glands contained abscesses. No changes were observed in the cavity of the mouth and its mucous membrane. There were no lesions in the throat organs. According to information received the sheep had not shown on section any other morbid changes. The pus in all the abscesses was exceedingly viscous. It was nearly impossible to distribute it in preparing the cultures. In the prepared cultures growths of *B. purifaciens* were obtained.

THE CHARACTERS OF THE ISOLATED BACILLUS.

From all the six cases recorded above there was thus obtained a species of bacillus, which was the only one present as shown by both microscopical and cultural examinations.

Morphology and Occurrence.

In microscopic examination of the viscid pus, which consisted of leucocytes with decomposed nuclei and only a few normal living pus cells, the organism could be discovered only with some difficulty. The bacillary forms are seen as *Gram-negative short rods*, situated singly here and there among the degenerated leucocytes. In the majority of cases several fields had to be searched before the organism could be shown. In cultures on agar or coagulated serum they were oval or almost spherical in form. In bouillon they appeared as distinct rods and often in chains of 6 to 10 elements. They lacked spores and were non-motile.

Cultural Characters.

Serum-Agar Plates. Already after twenty-four hours' incubation at 37° C. colonies could be plainly seen. The deep colonies attain a size of 0.4-0.5 mm. and the surface colonies 0.8-1 mm. After a further twenty-four hours the surface colonies reach a diameter of 2.2-5 mm. They are intensely bluish in colour and iridescent. When examined under the low power of the microscope they show no characteristic structure. The deep colonies are lenticular in shape while the surface colonies are quite round with even margins. They show a loose consistency and are easily broken up with a platinum needle.

Agar-slant Cultures do not exhibit any special characteristics either. After twenty-four hours' incubation there appear thin, iridescent, bluish colonies which soon congregate into a diffuse layer. The condensation water is cloudy and after some time a sediment is observed to form at the bottom of the tube without any clearing of the upper layer of the fluid.

In *Stab Agar* a vigorous "nail culture" was obtained, with a uniform growth on the surface and along the line of inoculation.

In *bouillon* a diffuse sediment is formed, which partly sinks to the bottom, although the rest of the medium does not become clear. No surface pellicle is formed. The addition of serum increases the growth to a perceptible extent, but does not cause any diffuse sediment and yields an abundance of small clumps or colonies, which for the most part settle on the walls of the tube and gradually sink to the bottom, the medium becoming then quite clear.

On *gelatine* the growth is very slow, there being no distinct growth before ten days' incubation at 18° C. A nail culture is obtained. No liquefaction takes place.

On *Löffler's Serum*, on a mixture of three parts horse serum and one part 10 per cent. dextrose bouillon, which has been allowed to coagulate, the bacillus grows quite vigorously and forms hemispherical, succulent colonies, which are devoid of colour pigment and are easy to pick off. The water of condensation is diffusely cloudy without any gas bubbles and with a vigorous deposit at the bottom.

In *milk* hardly any growth appears and the medium does not coagulate.

On *potato* there is a rather feeble growth. The colonies are succulent and greyish-white in colour.

No hæmolysis is produced in *blood-agar plates* and there is no colour change.

In ten days' bouillon cultures neither indol nor sulphuretted hydrogen could be shown.

Fermentation tests with *carbohydrates* showed acid formation but no gas in a number of the test substances.

Dr. Martin Kristensen of the Royal Serum Institute, Copenhagen, has very kindly controlled my fermentation tests and supplied the following information: Two different strains, 506/26 and 578/26, were sown in separate series. The medium employed was bouillon containing 1 per cent. Liebig's meat extract, 1 per cent. Witte peptone, and $\frac{1}{2}$ per cent. common salt, with 1/400 per cent. Bromothymol blue as indicator. To this mixture was added $\frac{1}{2}$ per cent. of the sugar solutions in question (Pfahnstiehl Chemical Comp.) and sterilisation was effected by ten minutes' heating up to 100° C. The tubes were allowed to stand in the incubator for fourteen days and the reaction was read off daily. The purity of the cultures was controlled in various ways in the course of the tests. The results were as follows:—

		<i>Erythrol</i> X	<i>Adonitol</i> X	<i>Dulcitol</i> X	<i>Sorbitol</i>	<i>Mannitol</i> X	<i>L-arabinose</i>	<i>Xylose</i>	<i>Rhamnose</i> X	<i>Maltose</i>	<i>Lactose</i> X	<i>Saccharose</i> X	<i>Raffinose</i> X	<i>Inulin</i> X	<i>Salicin</i>
506/26	...	14 O	14 O	14 O	14 O	1 S	14 O	1 S	14 O	1 S	6 S	1 S	14 O	14 O	14 O
278/26	...	14 O	14 O	14 O	14 O	1 S	14 O	1 S	14 O	1 S	6 S	1 S	14 O	14 O	14 O

The fermentation test was carried out at a temperature of 37° C. The tubes with the sugar solutions marked X were fitted with an inner inverted glass tube for the collection of gas. No gas production was observed at any time.

S = complete yellow colouring of tube within twenty-four hours.

6-14 = yellowish-green colouring after six days ; even after fourteen days the colour had not become entirely yellow.

EXPERIMENTS ON ANIMALS.

Guinea-pigs were inoculated intramuscularly, subcutaneously, intracardially, and intraperitoneally with doses of 0.5-5 c.c. bouillon cultures. No morbid changes occurred. The result was the same when an emulsion of pus was injected.

Rabbits were insusceptible to intravenous, intraperitoneal, and subcutaneous inoculation.

Mice were not susceptible to subcutaneous injections. Test animals inoculated intraperitoneally with a large dose (0.5 c.c.) died within twenty-four hours. In the abdominal cavity only an increased quantity of fluid was met with. The bacteria could be isolated from the heart's blood. A dose of 0.1 c.c. bouillon culture injected intraperitoneally did not prove fatal.

Two *chickens* were inoculated intramuscularly with 0.5 c.c. bouillon culture and also proved to be insusceptible.

Two *horses* received subcutaneously in the neck 1 c.c. and 5 c.c. bouillon culture respectively. Both animals developed a painful œdema at the site of inoculation, which attained an area as large as the palm of a man's hand. On the third day after inoculation there arose in the centre of this œdema an abscess as large as a walnut, containing a rather thin, serum-like secretion. Cultures were prepared from the contents of the abscess and the injected bacillus was obtained in pure culture. The temperature had remained normal all the time.

A *cow* was injected in the same manner with 1 c.c. The culture disappeared entirely without the formation of any abscess.

A *pig*, three weeks old, received 1 c.c. bouillon culture subcutaneously. No reaction occurred at the point of inoculation.

A *dog* also was not susceptible to subcutaneous injection.

A *he-goat* received 2 c.c. subcutaneously in the neck. On the second day an abscess formed at the site of inoculation and it burst open on the sixth day.

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SHEEP.

The point of greatest interest was, of course, how the tests on sheep would turn out. To carry out these experiments two animals were procured, a lamb born late in the year, weighing 21 kilos, and a ram one-and-a-half years old, weighing 53 kilos. The lamb was inoculated intravenously on the November 18th, 1918, with 1 c.c. bouillon culture. On the 20th the general condition seemed disturbed and the temperature had risen to 40° C. These symptoms disappeared, however, in two days, but on November 30th a hard swelling was observed at the point of inoculation. Nearly the whole of the vein felt thrombosed, and when incised it emitted a thick secretion which both microscopically and culturally showed the presence of the injected

organism in pure culture. There seemed to be nothing amiss with the lamb later on. It was killed on January 25th, 1919, but showed on section no morbid changes except *two pea-sized abscesses in the right lung*. The bacillus was recovered from these abscesses. The bronchial lymph glands showed no changes.

The ram was inoculated subcutaneously on November 18th, with an emulsion of a twenty-four hours' culture on Löffler's serum. The temperature rose from 39° C. to 40·2° C. On November 26th an induration as large as a walnut was found at the point of inoculation. This was fluctuating on November 30th and was then opened. The contents of the abscess consisted of a thick, viscid secretion teeming with bacteria, partly *Bac. pyogenes* and partly the organism injected. When the ram seemed to have recovered completely it received on December 14th an injection of 10 c.c. bouillon culture intraperitoneally. It showed no further signs of reaction and began to put on flesh, weighing 61 kilos when killed on April 14th, 1919. There was now no trace of the first subcutaneous inoculation. From the second injection, administered intraperitoneally, there was found in the abdominal cavity, exactly opposite the site of inoculation, an abscess as large as a man's fist, which was surrounded by a fibrous capsule. The reticulum adhered firmly to this abscess. The peritoneum presented no changes. Neither did the organs of the abdominal cavity except the lymphatic glands of the liver. These were enlarged and contained *multiple abscesses as large as hazel-nuts*. They showed a rather thick, fibrous capsule and contained a thick, doughy, green pus that emitted no odour. In the chest cavity several abscesses varying from the size of a hazel-nut to that of a walnut were encountered in the posterior mediastinal lymphatic glands. One abscess was 10 cm. long by 3 cm. wide. The rest of the lymphatic glands and the pleura contained nothing of note. In the posterior lobe of the left lung *one hazel-nut-sized abscess and one pea-sized* were found in the parenchyma. All the abscesses contained viscid, inodorous pus. No bacteria could be seen microscopically, but in the cultures prepared some thirty colonies of the same kind, identical with those injected, were obtained.

While these experiments were being conducted one of the ewes at the laboratory had given birth to a lamb on January 21st, 1919. On February 14th, when three weeks old, this lamb was inoculated with 5 c.c. of a forty-eight hour's bouillon culture from a suspension of the original stock culture from October, 1918, which had not passed through any experimental animal. Even this young lamb did not seem to suffer any serious injury from the test, but continued to grow in weight and was in good condition when killed on April 10th, that is, more than two months after the inoculation. At the point of inoculation in the abdominal cavity there was *an abscess as large as a hen's egg, containing green, viscid secretion*, which had no smell. This secretion did not run out when the abscess was punctured owing to its doughy, coherent consistency. Encapsulated in *the reticulum were six such abscesses of varying size*. The majority were as large as walnuts. In the psalterium there were four abscesses. Between the liver and

the diaphragm there were four, the greater part of one of them being situated in the parenchyma of the liver. In the hilus of the spleen there was observed one such abscess that was at large as a hen's egg and weighed considerably more than the spleen itself. In the lymphatic glands of the liver were five, most of them as large as hazel-nuts. Two were encountered in the pelvic lymph glands and one in the wall of the colon, the latter opening towards the intestinal lumen. The organs of the chest cavity also showed changes, there being a *hazel-nut-sized abscess in the posterior lobe of the left lung*. All abscesses were of the same type, with viscid, plastic pus. No bacilli could be shown microscopically but they were obtained by cultural tests. *Bact purifaciens* was obtained in pure culture.

On April 8th, 1921, a feeding experiment was made on a lamb, 20 c.c. bouillon culture being introduced by means of a tube direct into the rumen. No changes were apparent when the lamb was killed three months later.

On November 19th, 1921, 3 c.c. bouillon culture was injected into a full-grown sheep through *the milk duct of the right half of the udder*. In a week's time a painless induration, the size of a hen's egg, was observed in the udder. This increased more and more in size, so that by November 21st it had grown twice as large as a man's fist. The udder secretion had been examined before the inoculation for cellular constituents and bacteria, but nothing noteworthy could then be shown. Two weeks after the injection an abundance of polynuclear cells was met with but very few bacteria. On February 1st, 1922, the sheep began to have some difficulty in getting on its legs. At the base of the teat of the injected half a couple of fistulæ were observed, which emitted a ropy, thick pus. On February 9th the sheep, being unable to get up and in a very emaciated condition, was killed.

Post-mortem examination showed that this ewe was pregnant with three almost fully developed fœtuses. The left half of the udder *was morbidly changed and had about ten variously-sized, thick-walled abscesses, containing viscid, green pus*. Three of these abscesses opened out into fistulæ at the base of the teat. The lymphatic gland of the right udder was as large as a walnut and contained an abscess. *The right loin gland was greatly enlarged, being about the size of a hen's egg and containing six abscesses*. No foci or abscesses were encountered in the lungs, liver, kidneys, or lymphatic glands. The kidneys were greatly degenerated. Otherwise nothing worthy of note was met with. Bacteriological examination of the pus from the different foci showed *Bact. purifaciens* in pure culture. Cultures from the blood yielded no growths.

In Cases 4 and 5 several inoculation experiments were also made subcutaneously on sheep with pus as well as with pure cultures. The sheep reacted with a rise in temperature of 1°-2° C. and with a typical abscess at the point of inoculation.

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It thus appears that the isolated bacillus in pure culture possesses the power to cause similar pyogenic processes to those shown in the

spontaneous cases. It is not very virulent but produces local foci with metastasis here and there and readily finds access to the lymphatic glands. No septicæmia, with enormous quantities of the bacteria in the blood channels, has ever occurred in the inoculated sheep.

AGGLUTINATION TESTS.

Rabbits were inoculated intravenously with bouillon cultures, 5 c.c. killed cultures on May 12th, and 2 c.c. living cultures on May 18th, May 27th, and June 1st. The titre then proved to be 1 : 1,000. The agglutination antigen consisted of emulsified slant agar cultures diluted with 0.5 per cent. phenol. Difficulty was sometimes experienced owing to the occurrence of spontaneous agglutination. Some of the naturally infected sheep had a titre varying from 1 : 320 up to 1 : 1,280. Healthy sheep agglutinated in a dilution of 1 : 20, sometimes higher, but not higher than 1 : 100. Serum from the ewe shown in Fig. 2 agglutinated in a dilution of 1 : 1,280, but not in 1 : 2,560. The Ewe in Fig. 1 agglutinated in a dilution of 1 : 640. This ewe's lamb agglutinated when fourteen days old in a dilution of 1 : 320. A ewe, in which a subcutaneous injection of 10 c.c. bouillon culture resulted in a local abscess formation, gave two months later an agglutination in a dilution of 1 : 640. A healthy control ewe showed at the same time a titre of 1 : 20.

* * * * *

We thus see that here we have cases of pus formation in sheep which readily becomes localised in the subcutis and the lymphatic glands as well as in the internal organs. A characteristic of all five cases where the head was sent in for examination was that numerous abscesses were present in the cheeks, lower jaw, and around the pharynx.

The associated lymphatic glands have also shown abscesses at the same time. The kidney, liver, and udder have also been the seat of lesions in one or two of the cases. According to Christiansen's observations and mine, it seems as if abscess formation in the lungs was the commonest of the internal complications accompanying the infection. It should be noted, however, that there is never any question of broncho-pneumonia, but only isolated foci, which encapsule themselves. In this the disease differs from pyobacillosis, where a rather diffuse, compact induration of the lung with necrosis and disintegration is usual. It is a noteworthy fact that the musculature and the true muscular lymphatic glands have in no case whatever been attacked, neither have any joints or tendon sheaths. The pus has shown a very characteristic appearance, having been viscous, almost gummy, and plastic. It has not been at all like the pus generally found in abscesses in other kinds of animals. Therefore the denomination caseous lymphadenitis does not suit here. The two cases described last showed certain peculiarities meriting special emphasis. Case 5, for instance, showed a chronic, proliferative peritonitis, which reminded one very much of

the tuberculous nodular tumours in the peritoneum of cattle, a peculiarity not present in any of Christiansen's cases. Case 6 had more of the character of a pustular dermatitis and was not so deep-going as is usually the case.

The affection has not been observed in lambs but only in full grown sheep and has manifested itself in all cases except one while the animals were being kept indoors, *i.e.*, from January to April. The disease has been non-malignant in so far as it has not spread among the flocks to any extent. The virus belongs to the typical pus-producers and has a very slow effect. It does not therefore give rise to any acute affliction, but only chronic. It spreads *via* the lymphatics and produces metastasis. Death from bacteriæmia does not occur. As the abscesses, however, are sometimes present in such large numbers and sizes as to interfere with the functions of the respective organs, death can easily occur. It may thus happen that the animals die owing to their lips, cheeks, and pharynx being destroyed, rendering it impossible for them to feed, or else the abscess secretion in the lymphatic glands of the pharynx and palatine curtain may press the air-passages together. If infection is present in the udder that organ seems to become entirely destroyed. The pathogenesis, with a chronic progress without any disturbance of the general state of health, readily calls to mind that of pyobacillosis, as it sometimes appears in pure infection with *Bac. pyogenes*. A fact worthy of note is that all the cases examined were cases of pure infection. Mixed infection with *Bac. pyogenes*, cocci, streptococci, or other commonplace pus-producers, has never been shown in the closed abscesses, whether these were situated in the organ itself or in the lymphatic glands. Finally, it has been observed that a characteristic common to all the cases above cited was that the specific bacillus was found very sparingly in the pus and that its presence could in most cases only be discovered after transplantation on the various culture media.

Christiansen's investigations concerning this disease were based on material received from one and the same sheep-farm, where Veterinary Surgeon *Bech* had had several cases of pus-formation in the head under treatment. In a flock of a hundred sheep a few of the animals had been infected after the house-feeding had begun; some, however, recovered. About ten animals in all died during the winter. A bacteriological examination was made of a few lungs and two whole carcasses. The symptoms were: swelling of the submaxillary and subparotideal lymphatic glands and a little running at the nose. After some time the glands suppurated. The animals ceased to eat and died in a cachetic state. The duration of the disease in fatal cases was about two months. In the lungs and their lymphatic glands sent in were large abscesses containing a homogeneous, very viscous, inodorous, greyish-yellow mass of pus. In one of the sheep ulcers and abscesses were found in the lower lip and in the jaw-cavity. The associated lymph glands were morbidly changed by abscesses. In both lungs there were five or six abscesses from the size of a pea to that of a bean. The other sheep also exhibited an abundance of

subcutaneous abscesses in the head and in the lungs. Abscesses were also seen in the lymphatic glands of the head. In addition there was a bean-sized abscess behind the ethmoid bone close to the brain cavity. From all the cases there was isolated a Gram-negative, non-motile, oval bacillus, the cultural properties of which were described in detail. The description agrees in all essentials with what I found later in my cases. The organism did not show itself to be pathogenic for the usual test animals, with the exception of mice provided they were inoculated with a large dose intraperitoneally. A he-goat received 0.2 c.c. bouillon culture subcutaneously, and although it became feverish it survived and showed no abscess formation. A she-goat injected subcutaneously also proved to be insusceptible, getting only a large painful swelling at the site of inoculation but no abscess.

With regard to the origin of the disease, it may be assumed that the virus enters the organs through wounds on the lips or in the mucous membrane of the mouth cavity. It is more than probable that the bacillus in question belongs to the normal flora of the mouth and finds its way into the underlying tissue by means of lesions caused by spiny plants that form part of the animal's food. Christiansen mentions that in the coastal district where his cases had occurred it was suspected that a very common prickly plant, the *Hippophæ rhamnoides*, was the cause of the disease. A point worthy of note is that three of the four cases examined by me were animals belonging to flocks that had had access to pasturages along the seashore. No particular spiny-leaved plant has been suspected, however. Moreover, the disease first manifested itself during the winter feeding and on none of the farms were the animals fed with hay from any foreshore meadow. Now it is possible that the mucous membrane had already been injured during the preceding autumn, and that the pus-formation had been going on furtively all the time, an assumption that agrees very well with the chronic course of the affection in inoculated test sheep.

Disregarding cases in which commonplace cocci and streptococci and an occasionally tubercle bacilli cause abscesses in sheep, it is really only pseudo-tuberculosis and pyobacillosis that can be confused with the form of infection under discussion. In pseudo-tuberculosis, or so-called caseous lymphadenitis in sheep, a disease I have never come across in Sweden, it is the lungs and their lymph glands, the muscular lymphatic glands, and sometimes the udder, that are morbidly changed. The abscesses in the lymphatic glands are surrounded with a capsule and contain a smeary, caseous, greenish-yellow secretion that sometimes dries up and then shows a stratification of the purulent mass like the coats of an onion. In the pus there are masses of Gram-positive bacteria, which are not serophilic and do not peptonise coagulated milk and which keep their vitality for several months without being transplanted. It produces acid in only a few carbohydrates and is pathogenic for guinea-pigs. The disease has been shown in different countries; by Cherry and Bull in Australia, by Gilruth in New Zealand, by Nørgaard and Mohler in North America, and by Noack and Glässer in Germany. In France Carré ascertained

that the bacillus can assume various forms differing from that originally described by Preisz and Nocard. Still, the characteristic qualities of the organism is such that it can easily be distinguished from both *Bac. pyrogenes* and *Bact. purifaciens*.

In pyobacillosis, which is not so rare, manifesting itself as a rule in isolated cases in different herds, pus-formations are met with in the subcutis, the joints, the lungs, and the udder. *The lymphatic glands are less frequently affected.* Olt has described cases in Germany and the present writer has observed it several times in Sweden. Etiologically and clinically it is like pyobacillosis in cattle. The pus is thick and light green in colour. The bacillus is Gram-positive and is found in great masses in the pus. It is serophilic and dies off in the cultures in two or three weeks unless transplanted. It peptonises coagulated serum and coagulates milk; produces hæmolytic in blood-agar plates and forms acid in several different carbohydrates. As regards guinea-pigs it is practically avirulent. It is identical with *Bacillus bovis et suis* as described by Künnemann, Grips, Glage, Holth, Brown, Orcutt, Berger, and others. Carré found pus formations as a complication of agalaxia in sheep, but the organism he isolated was different in many respects. Thus, he mentions that it did not grow well on coagulated serum, and that it was pathogenic for guinea-pigs but not for calves. Descazeaux has given an account from Chile of typical pyobacillosis in sheep. His bacillus most closely resembled Carré's but grew excellently on coagulated serum and peptonised that medium. He also states that it did not split up carbohydrates. Yet it was highly pathogenic for guinea-pigs. Unfortunately the description of the cultural properties given by these two investigators is not exhaustive enough to allow a comparison on all points with *Bac. pyrogenes* in cattle and swine. At all events they are organisms very closely related to *Bac. pyrogenes* and Preisz-Nocard's bacillus and easily distinguishable from *Bact. purifaciens*. In conclusion mention should be made of a form of infection with pus-formation in sheep that has recently been described by Spray from America. When studying the lungs of sheep at some abattoirs in America he found that the lymphatic glands in the thoracic cavity were often morbidly changed by abscess-formation with an accumulation of green, cream-like or caseous secretion inside a connective tissue capsule. At the same time the lungs showed a chronic inflammation with necrotic foci. The pus contained an abundance of fine, Gram-positive rods. In the first cultures prepared on blood-agar it grew like a rod but assumed a diplococcic form in continued culture tests. He therefore assumed that this was its typical form. He took it for granted that the Preisz-Nocard bacillus was identical with *Bac. pyrogenes bovis et suis*, a point of view that Dunkel also considered he had arrived at, chiefly by serological experiments, and he carried out a comparative examination of the bacillus found by him and the Preisz-Nocard bacillus.

TABLE I.—ESSENTIAL DIFFERENCES BETWEEN SOME BACTERIA PATHOGENIC FOR SHEEP.

	Morphology Staining.	Plain agar.	Serum agar	Blood agar.	Infusion broth.	Gelatine.	Milk.	Löffler's serum.	Carbohydrate. Lactose. Saccharose. Xylose. Maltose.	Pathogenicity tip for guinea- pigs.
<i>Preisz-Nocard bacillus.</i> Causes suppurat.	Gram — rod. pleomorphic. Non motile.	Abundant, white brittle colonies.	Abundant, yellowish, dry.	Abundant, 5 mm. in dia- meter. No hemolysis.	Turbid, pel- licle, granu- lar sediment.	No liquefac- tion.	No change.	Abundant. No lique- faction. Yellowish tinge.	No gas. No acid.	Fatal orchitis.
<i>Bacillus Pyogenes.</i> (Grip's and Kirm- man's bac.) Causes suppurat.	Gram + rod. pleomorphic. Non-motile.	No growth.	Scanty growth.	Scanty colo- nies 0.5 in mm. dia- meter. Hemolysis evident.	No growth.	Liquefaction.	Coagulum rapidly pep- tonised.	Abundant liquefaction.	Acid formed. No gas.	Abscesses.
<i>Bacillus Pyogenes.</i> (Des- cazeaux). Causes sup- purat.	Gram + rod. pleomorphic. Non-motile.	Scanty growth.	Small colonies.		Scanty growth.		Coagulum rapidly pep- tonised.	Abundant liquefaction.	No acid.	Fatal.
<i>Bacillus Pyogenes oris.</i> (Mal de Lure, Carré). Causes suppurat.	Gram + rod. pleomorphic. Non-motile.	Scanty growth.	Scanty growth.		No growth.	Scanty growth.	Coagulum.	No growth.		Fatal orchitis.
<i>Diphtheria</i> (Spray). Causes suppurat.	Gram + rod. in pus and blood-agar. pleomorphic. Diplococcus. in old cul- tures. Non- motile.	No growth.	Small colonies.	Small colo- nies 0.5 mm. in diameter. Hemolysis.	No growth.	Liquefaction.	Coagulum rapidly pep- tonised.	Abundant liquefaction.	Acid formed. No gas.	Fatal; necro- tic areas in the liver.
<i>Bact. Parvifaciens</i> (Chris- tiansen). Causes sup- purat.	Gram — rod. Non-motile.	Abundant growth.	Abundant growth.	Abundant growth. No Hemolysis.	Turbid.	Scanty growth. No liquefaction.	No change.	Abundant growth. No liquefaction.	Acid formed. No gas.	No changes.
<i>Haemophilus oris</i> (Mit- chell). Causes pneu- monia.	Gram — short rod. Non- motile.	No growth.	Scanty growth.	Abundant growth. No hemolysis.	No growth.	No liquefaction.			Acid formed. No gas.	Fatal sep- ticemia.
<i>Pasteurella.</i> Causes pneumonia.	Gram — short rod. Non- motile.	Abundant growth.	Abundant growth.	Abundant growth. No hemolysis.	Abundant growth.	No liquefaction.	No change.	Abundant growth. No liquefaction.	Acid formed in saccharose No acid in xylose, lac- tose, maltose	Fatal septi- cemia.

TABLE II.
FERMENTATION REACTIONS OF SOME BACTERIA PATHOGENIC TO SHEEP.

	<i>Preisz-Nocard bacillus (Spray)</i>	<i>Bac. pyogenes ovis (Magnus- son)</i>	<i>Diplococcus (Spray)</i>	<i>Bac. purificans Christiansen (Magnusson)</i>	<i>Hemophilus ovis (Mitchell)</i>	<i>Pasteurella ovis (Magnusson)</i>	<i>Bact. erysipelat ovis. (Christiansen)</i>
Fructose ...	+	+	+	+	+	+	+
Galactose ...	+	+	+	+	+	+	+
Glucose ...	+	+	+	+	+	+	+
Mannose ...		+		+	+	+	+
Arabinose ...		—	—		+	—	—
Xylose ...	—	+	+	+	+	—	—
Rhamnose ...		—		—	—	—	—
Lactose ...	—	+	+	+	+	—	+
Maltose ...	—	+	+	+	+	—	—
Saccharose ...	—	+	+	+	+	+	—
Raffinose ...		—		—	+	—	—
Amyl. sol. ...		+		—			
Inulin ...		—		—	—		
Salicin ...	—	—	—	—	—		
Glycerol ...	—	—	—	—		—	—
Erytrol... ..		—		—		—	
Adonite ...		+		—		—	—
Dulcitol ...		—		—		—	—
Mannitol ...	—	—	—	+		+	—
Sorbitol ...	—	—	—		+	+	—
Inosite... ..	—		—	—	—	—	

+ = acid formed, no gas. — = No change. Blank = not investigated.

In order to get good growth 10 per cent. sterile horse-serum was added. Kindly controlled by Dr. E. Klarin.

Spray procured cultures of the Preisz-Nocard bacillus from three different sources: Pasteur Institute, Bureau of Animal Industry, and British National Collection of Type Cultures. These stocks were identical among themselves but represented quite a different species of bacillus from the one Spray believed he had discovered. It is necessary, however, to study the synoptical table he has given of his examinations to see that his so-called diplococcus is closely related to *Bac. pyogenes*. In pus from lymphatic glands and lungs it appeared as a Gram-positive rod, likewise on blood-agar. In milk and serum bouillon it resembled a coccus and formed itself into chains. This pleomorphism is quite typical for *Bac. pyogenes*. Jowett and also Brown and Orcutt have especially pointed out that streptococcic forms are by no means uncommon. Moreover, it had the power to peptonise coagulated serum and to coagulate and peptonise milk. In blood-agar plates it gave hæmolysis. Even the fermenting reactions and manner of growth in other media were partly characteristic. He examined eleven different strains and their main characters appear from the accompanying synoptical table. Spray has in his paper conclusively demonstrated the great difference between the Preisz-Nocard bacillus on the one hand and *Bac. pyogenes* on the other, and he has at the same time shown that in North America *Bac. pyogenes* causes chronic necrotising pneumonia and purulent lymphadenitis in sheep.

The nomenclature employed by the present writer for the different bacteria is that commonly used in Sweden and which has been suggested by or after the respective authors. The name *Hemophilus* (*see* Table I) signifies that the bacillus in question is a Gram-negative, non-motile organism, growing only in the presence of hæmoglobin or some other body fluid, and closely related to Pfeiffer's *B. influenzae*. The name *Bacillus pyogenes* should be changed to the designation *Corynebacterium pyogenes*, as in spite of its peptonising effect on serum and milk it is closely allied to the Diphtheroid Bacilli group with its pleomorphic, Gram-positive species.

SUMMARY.

A pyæmic disease, localised in the subcutaneous soft parts of the head and the associated lymphatic glands, has been met with in four different flocks of sheep. In certain cases abscesses have also occurred in the lungs, the liver, the kidneys, and the udder. The abscesses contained a plastic, viscous pus, in which were present small numbers of Gram-negative rods in pure culture. These were identical with *Bac. purifaciens* Christiansen.

In none of the cases could the Preisz-Nocard bacillus, *Bact. pyogenes ovis* (Carré and Descazeaux), or Sprays's diplococcus be isolated or shown. The last mentioned seems as regards its cultural properties to be identical with *Bacillus pyogenes*. Spray mentions that in the pus from the abscesses it appeared as a Gram-positive rod, while in serum broth it assumed the form of a diplococcus. This tallies very well, for *Bac. pyogenes* is highly pleomorphic. The disease is not transferable from animal to animal, but seems to be contracted through wound infections in the mouth cavity caused by a certain spiny plant or plants that the animals eat when grazing in pasture land along the sea-shore. The organism is Gram-negative, non-motile, with an optimum growth at a temperature of 37° C. It does not coagulate milk. It produces acid but no gas in fructose, galactose, glucose, mannose, mannitol, xylose, maltose, and saccharose. Slight acid production occurred in lactose, but only after six days. Blood serum of infected sheep agglutinates in a dilution of 1 : 1,280. It is pathogenic for white mice inoculated intraperitoneally and for goats and horses injected subcutaneously. It is pyogenic for sheep when injected subcutaneously, intravenously, intraperitoneally, and intramammarily. Guinea-pigs, rabbits, and other test animals were not susceptible. From the literature it appears as if this disease has been described only once before, viz., in 1917 by Christiansen in Denmark. It has also been shown at the State Veterinary-Bacteriological Institute at Stockholm in 1927.

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A BACTERIOLOGICAL INVESTIGATION OF CASES OF TUBERCULOSIS IN EQUINES.

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TUBERCULOSIS in the domestic animals other than bovines and swine is not of great economic importance; it is, however, of interest to the pathologist to determine the distribution and characters of the lesions met with in the various species and the exact characters of the causal bacilli. In domestic pets in such close contact with human beings as is frequently the case with dogs and cats it is especially important to determine to which of the groups, human or bovine, the causal organism belongs. Again, many strains, particularly some of those from equine sources, are distinctly interesting in that they show anomalous characters.

The work here reported has been carried out during the past two years, but many of the strains were isolated in 1926 by Dr. W. H. Andrews; I am greatly indebted to him for these and for the post-mortem notes and history of the animals from which they came.

From fresh material an attempt was always made to obtain a direct culture, either without further treatment or after emulsification with sterile saline. Guinea-pigs and, in some cases, rabbits were inoculated subcutaneously, and in event of failure of direct culture a strain was raised from a guinea-pig. The primary inoculation of rabbits also gave a preliminary idea of the virulence for that animal.

Contaminated Material.

Various methods of dealing with contaminated material have been tried.

Antiformin was used in a few of the earlier cases. Treatment with a 20 per cent. actual concentration for half-hour was adopted as a standard method, but on two occasions material was subjected to a 15 per cent. concentration for periods ranging from ten minutes to two hours. All emulsions were, after treatment, immediately diluted at least eight times with saline, centrifuged at high speed, washed twice, and sown on suitable media. It was found that with the sample of antiformin used results were very erratic, *e.g.*, in one case in which material moderately rich in acid-fast bacilli was sown before and after treatment quite numerous colonies were obtained with the original material on three tubes out of six, whereas no growth was obtained from the treated material. Moreover, the latter failed to infect three animals inoculated with it, although the virus was later found to be fully virulent for rabbits and guinea-pigs. Further, the antiformin did not always prevent the growth of contaminating colonies. The results obtained with this antiformin may have been unusual, but were sufficiently disturbing to cause a better method to be sought.

Petroff's Method.

Petroff studied the effect of various dyes on the development of tubercle bacilli and other organisms; he found that gentian violet checked the growth of contaminating organisms more satisfactorily than any other agent which he tried and yet did not seriously affect the tubercle bacilli. Many streptococci and staphylococci were, however, not inhibited, and their growth was prevented by an initial treatment with 3 per cent. KOH for half-hour at 37° C. According to his technique the material after the preliminary alkali treatment is made neutral with HCl, then centrifuged and the deposit sown on a medium made up with egg and double strength broth, containing 5 per cent. glycerine and sufficient gentian violet to give a 1 in 10,000 final concentration.

Own experiments.—Broth egg medium was prepared and divided into two portions, to one of which glycerine was added. Plain egg and 5 per cent. glycerine egg were also prepared, without broth, from the same batch of eggs. The broth egg medium as recommended by Petroff gave on the whole a better growth than parallel plain media. It was also noted that, although the medium was prepared in exactly the same way and the same percentage of gentian violet was added, the colour of the final product varied considerably. The cause of this was not obvious and had no manifest effect on the value of the medium.

In most cultivation experiments the same suspension of material containing tubercle bacilli was, after treatment with 3 per cent. KOH, sown on media of the same batch, without the gentian violet as well as on those with it. It soon became evident that the treatment with alkali was of far greater value than was the effect of gentian violet, which appeared to retard not only other organisms but also the tubercle

bacilli. In consequence the following simple method for obtaining primary cultures from contaminated material was adopted with useful results.

If it was found that the only seed material was contaminated or that the cultures sown from it were showing contaminating colonies, a little of the original material was taken and carefully mixed or, if solid, well triturated in a mortar with an equal volume of 6 per cent. KOH for ten minutes. A small amount was then rubbed over the cultural media and the remainder placed in a water-bath or incubator at 37° C. Further slopes of medium were sown at varying periods up to six hours and even twelve hours. After a period generally under six hours, depending on its original character, the material begins to assume a mucoid consistency and is probably then in the most suitable state for sowing. It seems probable that the alkali has by that time penetrated all the particles and yet not been in contact with the tubercle bacilli sufficiently long to lessen seriously their chances of growth on artificial media or in inoculated animals.

CULTURAL MEDIA.

Primary cultures were almost invariably raised on egg or broth egg, with or without glycerine. At first serum and other media were tried but it soon became evident that egg was the most useful.

The differential media used in subsequent cultivation were plain egg, glycerine egg, broth egg, glycerine broth egg, serum agar, glycerine agar, plain serum, glycerine serum, plain potato, glycerine potato, glycerine broth, and occasionally plain broth. The percentage of glycerine used was always 5 per cent.

Egg Media.

Egg was found to have a serious drawback for subcultures as some strains, and in most cases rapidly growing strains, tended to die out very quickly. This was especially so if cultures were left in the incubator after they had passed their period of optimum growth. In an endeavour to discount this drawback 20 per cent. of a double strength broth (1 lb. of meat in 500 c.c. peptone water) was added to both the plain and glycerine egg. These preparations were used for subcultures in parallel with ordinary egg media. The addition greatly decreased the tendency to die out, and also favoured the growth of all tubercle bacilli without interfering in any way with the differential characters.

Media used for differential purposes were always strictly comparable in that they were made at the same time and from the same basal ingredients, the presence or absence of glycerine or other such agent being the only difference.

Besredka's Egg Medium.

It is stated by Calmette that there is a distinct difference between the characters of the bovine and human types when grown in Besredka's

medium: no such difference was observed between the strains described, all growth being of a fine or coarse granular character. Three avian strains tested did, however, produce a fine powdery type of growth.

The value of whole egg compared with yolk was tested, the two being separately made up, alkalised to the same degree, and then mixed in varying proportions. Samples of each were seeded but no difference was found in the character or amount of the growth produced by any of the strains used. Thus it appeared that whole egg could be used in the proportion of 1 in 20 of saline equally as well as yolk only, and it was found by comparative experiments to be advantageous to use only the amount of alkali necessary to produce a transparent medium.

Differential Subculture.

A full series of differential media was always sown as soon as possible after isolation, the seed material being a young culture from a strain which had been grown on media without glycerine.

Great difficulty was experienced in getting some of the more dysgonic strains to grow, but frequent subculture as soon as, and in some cases before, any growth was visible to the naked eye was successful as a means of invigorating them.

The cultural characters were determined on each of the different media at least twice, the seed being of different generations. If, as often happened, the results were doubtful, or for any reason unsatisfactory, the tests were repeated with the same or a subsequent culture. Two or more tubes of the same medium were sown at each test. The inoculum received by each tube of a series was approximately the same, the seed material being well emulsified in saline, distributed by means of a pipette, and then spread.

In order to get distinct colonies a platinum loop was used, and a number of tubes were sown in succession from a single loopful of inoculum; media containing glycerine were used for the odd numbers and those without glycerine for the even numbers, or *vice versa*. This method, though sometimes unsatisfactory, owing to a tendency of most of the bacilli to be left on the first or the first and second tubes, often gave useful results, the differences in the time before growth appeared and in the rate and character of growth being most marked.

Quantitative Inoculation.

The material used for measuring the virulence of a strain was always obtained from young cultures before the optimum growth period had been passed, it being estimated that cultures would then contain the maximum number of viable individuals. A eugonic strain was thus used for inoculation sooner than one that was dysgonic; most cultures were between one and three weeks old. In the earlier experiments cultures in Besredka's liquid egg medium were used, but later material from other media, such as serum, was employed. The alkaline egg medium was particularly useful for dysgonic strains.

All material was emulsified as finely as possible with saline and then allowed to stand for at least half-hour until all the coarser particles had settled. The supernatant was then decanted and a small quantity was immediately placed in carefully graduated narrow pipettes and centrifuged, the speed and time being always the same. The proportion of deposit to liquid was measured and the amount of bacilli in the emulsion calculated.

The actual weight was checked on many occasions by preparing a suspension containing a weighed amount and comparing it with the unknown suspension. This method, which is similar to that used by A. S. Griffith when working for the Royal Commission, enables one to measure the amount of bacilli present after allowing all but the finest particles to settle and so ensures that the doses, and particularly the lower ones, shall be accurately measured and consist of single bacilli and small clumps only, an important point when using the intravenous or intraperitoneal methods of inoculation.

Rabbits, which have been shown to give results parallel to those obtained with calves, were used as the differential animals. Guinea-pigs, and in some cases fowls, were also used.

In the earlier experiments four rabbits were inoculated with each strain; three received subcutaneous doses of 10 mg., 1 mg., and 0.1 mg. respectively, and one 0.01 mg. intravenously; three guinea-pigs received respectively 10.0 mg., 1.0 mg., and 0.1 mg. by the subcutaneous route. It was soon realised, however, that a single animal was not of much value for measuring the difference between the effect of doses varying in multiples of 10. Consequently in later experiments one rabbit received 10.0 mg. and one 0.1 mg. subcutaneously; one rabbit 1.0 mg. intravenously, and one 0.1 mg. intravenously; two guinea-pigs 10.0 mg. and two 0.1 mg. The guinea-pigs, although they only give confirmatory evidence of the nature of the strain, are a very useful control when the strain proves of very low virulence for rabbits or produces no lesions at all. Additional animals were often used, and in the case of a few cultures, obviously dysgonic, which the original inoculation had shown to be virulent for the rabbit, the 1.0 mg. dose intravenously was omitted.

The latter dose is very severe but, should it produce no tuberculosis or only chronic effects, it gives very strong evidence of the low virulence of the strain used; a culture of known human origin may in this dose produce severe effects, practically indistinguishable from those of a virulent bovine strain. 0.01 mg. is the most suitable dose for a single intravenous test; this amount of a bovine strain of normal virulence kills within forty days whereas such a dose has no permanent effect or only chronic effects if of human origin. The 10.0 mg. subcutaneous dose is of less severity than the 0.01 mg. intravenous, only producing chronic effects if the strain be a human one, but certainly killing if the strain be from a bovine, whilst the 0.1 mg. subcutaneous dose is the least severe; nevertheless, that dose of a normal bovine strain invariably kills or produces severe tuberculosis within three months.

The individual strains are described separately. Where the results are quite clear cut a brief summary and table only are given ; other strains are described more fully.

SYMBOLS.

The full post-mortem notes of each animal are not given, but where possible symbols are used. Complete particulars would entail needless repetition of the same well-known clinical pictures of tuberculosis in the rabbit and guinea-pig. The symbols A.T. (advanced tuberculosis), M.T. (moderate tuberculosis), and E.T. (early tuberculosis), refer to cases in which the disease has generalised in the way that is typical of a virulent strain and express the degree to which the disease had advanced. The term S.T. (slight tuberculosis) is used to indicate small lesions which are atypical in type or distribution, and so occur usually with strains of low virulence. The lesions produced by the latter were in certain animals too large or widespread to be correctly described as slight, and these cases have been designated M.T. or A.T. and further information given in footnotes.

During one of the epizootics amongst the guinea-pigs a large number of uninoculated animals died as well as many inoculated ones which showed early or moderate generalised tuberculosis lesions. Particulars were taken regarding the weights of the spleen and liver of normal animals and of animals dying at various intervals after infection. Each weight was expressed as a fraction of the body weight. It was found that the average weight of a normal guinea-pig's spleen was about $1/1,000$ th of the body, whereas diseased spleens varied from $1/500$ th to $1/50$ th, according to the time the animal had been infected. In some animals which survived 100-150 days after inoculation it was even higher. The liver of a normal animal forms about $1/50$ th body-weight, whilst after inoculation it may increase up to $1/5$ th and commonly reaches $1/20$ th.

CASE I.

The animal was a six years old cart gelding which was at work until about two months before death, when it was noticed to be losing condition. The animal fed moderately well but wasting continued, and about one week before death it was reported as definitely emaciated, with dyspnoea and a bilateral nasal discharge of a creamy viscid nature. There was a slight cough and dullness on auscultation. Symptoms became rapidly worse during the succeeding few days, until the animal was unable to rise and was destroyed.

Post-mortem Examination.

The mesenteric and colic glands were considerably enlarged and showed lesions of the usual character. The lungs contained miliary tubercles throughout and a large softened and apparently tuberculous tumour-like lesion was situated at the inferior border of the posterior lobe of the right lung.

Bacteriological Examination.

Smears made from the glands showed acid-fast bacilli to be present in very small number.

Direct culture was attempted but was unsuccessful ; the strain was obtained from a guinea-pig inoculated with material previously treated with antiformin.

Differential subculture showed this virus to be moderately dysgonic on all media. Growth on glycerine egg and glycerine broth egg ran parallel to that on the corresponding plain media ; but on glycerine agar, glycerine serum, and glycerine potato growth appeared to be often distinctly delayed and inhibited.

Virulence for rabbits was high. The delayed death of the animal which received 0.01 mg. intravenously may probably be correctly referred to the natural resistance of that particular animal ; at death lesions were of the kind typical of a bovine infection.

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Weight in grammes</i>		<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
	<i>Initial.</i>	<i>Final.</i>			
Guinea-pig. Rabbit ...			Antiformined mesent. gland.	Died 58 76 81	A.T. A.T. A.T.
<i>Quantitative tests with</i>		Besredka	culture 575, 845, and 979 days later.		
Rabbit ...			1.0 mgm. subcut.	Died 56	A.T.
			0.1	88	A.T.
			0.01 intrap.	107	A.T.
			0.01 intrav.	56	A.T.
Guinea-pig.	2000	1500	0.01 subcut.	34	M.T.
	650	470		72	A.T.
	645	500		82	A.T.
	540	500		126	A.T.
	565	430			

CASE II.

The spleen and lungs only of this animal were received. Both lungs were riddled with miliary lesions. The spleen was much enlarged and distorted and contained numerous tumour-like lesions varying from 0.5 cm. to 10 cm., the majority being small ; in many places there were large confluent masses. The lesions showed various shades of pale yellow and were usually firm or tough to cut, a slight grittiness being noticed in a few places. The centres of several lesions were softened and tubercle bacilli were extremely numerous in some of these, but equally scarce in others of the same type. They were very difficult to find in the firmer areas and in the pulmonary lesions.

Bacteriological Examination.

Contaminating organisms were numerous and no direct culture was obtained. Splenic material was emulsified, treated with antiformin, and used for seeding artificial media and for inoculating animals. The strain was obtained through a guinea-pig, which died fifty-eight days after inoculation, showing advanced generalised tuberculosis ; it was of definitely dysgonic type, growing about equally well on plain and glycerine media ; no growth was obtained on glycerine agar.

Inoculation of rabbits showed it to be of bovine virulence, all animals dying within the periods usually taken by a bovine strain and showing typical advanced generalised tuberculosis.

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
<i>Original inoculations.</i>			
Rabbit ...	Fresh spleen.	Died 60	A.T.
		64	A.T.
Guinea-pig.		58	A.T.
		69	A.T.
<i>Quantitative tests with Besredka culture 454 and 672 days later.</i>			
Rabbit ...	0.01 mg. intrav.	Died 26	A.T.
	10.0 mg. subcut.	Killed 104	A.T. (a)
	1.0	Died 62	A.T.
	0.1	83	A.T.
Guinea-pig.	10.0 subcut.	35	A.T.
	1.0	51	A.T.
	0.1	46	A.T.

(a) Chloroformed as it was in a dying condition.

CASE III.

The animal, a Hackney mare, was reported as having been losing condition for some time; anæmia and distinct stiffness in the cervical region had also been noticed.

Post-mortem Examination.

A piece of spleen with the splenic lymph gland attached were the only organs received. The lesions were of the characteristic tumour-like type and acid-fast bacilli were numerous.

Emulsified material from the lesions was treated with antiformin and used for culture and animal inoculations.

Bacteriological Examination.

Strains were obtained from a rabbit and two guinea-pigs; all were similar, definitely dysgonic and difficult to cultivate on glycerine media in the earlier generations. No growth was obtained on glycerine agar or glycerine potato.

Animal inoculations showed this virus to be of rather less than normal bovine virulence for guinea-pigs and markedly lower for rabbits. The rabbit which received 1 c.c. of an emulsion of the original material rich in acid-fast bacilli was noted at the time as particularly young and small; it died after forty-three days with lesions which showed that the virus was generalising but it is doubtful if they were sufficient to account for death. All the rabbits inoculated with weighed material remained alive at least twice as long as the average animal would have survived inoculation with the same dose of a standard bovine virus. The lesions found at the post-mortem examination of those inoculated subcutaneously were of a much more widespread and chronic type than is found after inoculation with a virus of high virulence, lung lesions in particular being less numerous and more irregular in size and kind. The animal which received 0.01 mg. intravenously died after 133 days, showing massive lesions in the lungs, kidneys, spleen, and glands quite different from those found after the same dose of a fully virulent bovine virus. The strain thus showed distinctly anomalous characters.

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
<i>Original inoculations.</i>			
Rabbit	Antiformined spleen and gland.	Died 43	M.T. (<i>young</i>)
Guinea-pig.		Killed 95	E.T. (<i>a</i>)
		96	E.T. (<i>b</i>)
<i>Quantitative inoculations with Besredka culture, 644 days later.</i>			
Rabbit ...	10.0 mg. subcut.	Died 147	A.T. (<i>c</i>)
	1.0	187	A.T. (<i>d</i>)
	0.1	253	A.T. (<i>e</i>)
	0.01 intrav.	133	A.T. (<i>f</i>)
Guinea-pig.	10.0 subcut.	32	M.T. (<i>g</i>)
	1.0	68	A.T. (<i>h</i>)
	0.1	147	A.T. (<i>i</i>)

(a) Local ulcer 1 cm. Inguinal gland enlarged and showing one small caseated centre; a few other glands enlarged only. Spleen had small tubercles up to 1 mm., liver up to $\frac{1}{2}$ mm.

(b) Two local ulcerated lesions about .5 cm. Spleen slightly enlarged, with a few tubercles up to 1 mm.; liver a few tubercles; lungs three small lesions.

(c) Lesions of chronic type, disease appearing to have been long in generalising and then slow in developing.

(d) Condition fair. Lesions widespread and of chronic type; about half right lung still crepitant.

(e) Condition poor, half lung crepitant, and other lesions very atypical.

(f) Half lung tissue out of action and beset with irregular type of lesion; lesions in kidneys, spleen, sacculus rotundus, cæcum, and all glands.

(g) Generalised lesions, but only moderately advanced and caseation not marked.

(h) Spleen dark and congested, not caseo-necrotic; liver of fibrous type, no caseous areas.

(i) Glands much enlarged and fibrous; spleen showed small number of fibrous lesions, liver small number of fibrous lesions, in part firmly caseous.

CASE IV.

The animal was a six years old cart horse. It was emaciated and showed marked stiffness of the cervical region and an enlarged pre-scapular gland. A typical reaction was obtained to tuberculin subcutaneously and intradermally.

Post-mortem Examination.

The spleen was enlarged three to four times and showed numerous, large, rounded, light-yellow, firm, and tumour-like lesions. The splenic glands were also somewhat enlarged. Only slight enlargement of the mesenteric glands. The liver was in a state of fine cirrhosis.

Bacteriological Examination.

Microscopic examination revealed no acid-fast bacilli and direct culture was not attempted. Material was emulsified and inoculated into two guinea-pigs and two rabbits.

Strains were obtained from all these animals and were similar and definitely dysgonic.

Quantitative inoculation of a guinea-pig strain showed that it was of moderate virulence for rabbits, all the animals either surviving longer than usual or showing lesions which were not typical of a virulent bovine virus.

It may be classed as a bovine strain of low virulence.

The slight effects produced in the animals which received original material support this view ; the number of bacilli received must have been very small but, had they been of virulent type, it is probable that they would have rapidly multiplied and overtaken any deficiency in numbers. Virulence appears to be a fact of greater importance than numbers.

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
<i>Original inoculations.</i>			
Rabbit ...	Emulsion of spleen, no acid-fast bacilli.	Died 133	S.T. (a)
Guinea-pig.		Killed 226	S.T. (b)
		Died 69	Local.
		Killed 75	S.T.
<i>Quantitative inoculations with Besredka culture, 600 days later.</i>			
Rabbit ...	10.0 mg. subcut.	Died 91	M.T. (c)
Guinea-pig.	1.0	106	M.T. (d)
	0.1	162	M.T. (e)
	0.01 intrav.	41	A.T.
	1.0 subcut.	42	M.T.
	1.0	89	A.T.
	0.1	104	A.T.

(a) Large local lesion, containing thin fluid with small flocculi. One lesion in left lung, two in right up to 2 mm.

(b) Small local lesion, both lungs showed scattered greyish-white nodules up to 1 mm. Both kidneys three or four greyish nodules about 1 mm.

(c) Moderate local lesion containing creamy caseous material ; iliac glands much enlarged and showing caseated areas. Lungs crepitant, each showing about twenty greyish areas up to 2 mm. and a few larger ones partly caseated and one with commencing calcification. Kidneys each two or three caseous surface lesions about 2 mm. ; bronchial gland and sacculus rotundus slightly affected.

(d) Large local lesion ; iliac, popliteal, bronchial, and renal glands enlarged and caseated. Lungs each about thirty greyish areas, with caseated centres up to 3 mm., but still crepitant in greater part. Kidneys each a dozen lesions up to 2 mm.

(e) Lungs half crepitant, showing moderate well defined lesions. Widespread disease of moderate character ; pericardium, ribs, and pleura affected.

CASE V.

This case is of particular interest in that the animal, a four years old Clydesdale gelding, was reported to have begun to lose condition two years previously and gradually to have wasted until it was destroyed, being then absolutely emaciated and unable to rise, though still feeding well.

Post-mortem Examination.

The spleen only was received. It was not enlarged but slightly distorted by about fifteen rounded light-yellow lesions, the larger ones being from 3 to 6 cm. in diameter ; many, however, were smaller and mostly situated around the periphery of the larger ones. The consistence of the majority was firm but two showed several softened areas.

Bacteriological Examination.

Acid-fast bacilli were moderately rich in one area but rare or absent in others.

A culture was obtained on egg from antiformed material and also from a guinea-pig; they appeared identical. Growth was always poor and particularly so on media containing glycerine. Tests in rabbits showed it to be of high virulence; one animal, however, which received 0.01 mg. intravenously died after twenty-one days showing small miliary lesions which did not obviously account for death, and a second lived fifty-one days but then died showing typical advanced lesions. On the other hand, the animals inoculated subcutaneously all died within eighty-six days, showing advanced generalised tuberculosis.

These results would not justify a conclusion other than that the strain was of normal bovine type.

ANIMAL INOCULATIONS.

<i>Animals.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
<i>Original inoculations.</i>			
Rabbit ... Guinea-pig.	Splenic material, very poor in bacilli.	Died 109 128	T. M.T.
<i>Quantitative inoculations with Besredka culture 529 days later.</i>			
Rabbit ...	10.0 mg. subcut.	Died 69	A.T.
	1.0	61	A.T.
	0.1	86	A.T.
	0.01 intrav.	21	E.T.
	0.01	51	A.T.
Guinea-pig.	10.0 subcut.	38	A.T.
	1.0	30	A.T.
	0.1	40	A.T.

CASE VI.

This strain was derived from the spleen of a Shire mare about which no history was available. The spleen was the only organ received and was typical in its appearance. It was enlarged and distorted by numerous rounded or oval tumour-like projecting lesions up to 8 cm. in diameter; the majority were firm, and of a light yellow colour and on section of homogeneous appearances, but a few of the larger lesions showed small softened centres, the central material varying in consistence down to thick creamy pinkish-yellow pus.

Bacteriological Examination.

No acid-fast bacilli were found by microscopic examination. Direct cultures all became contaminated, but the strain was secured from a guinea-pig inoculated with splenic material and chloroformed 175 days later. It showed generalised tuberculosis of a moderate degree.

Cultures were always of a dysgonic character, giving no evidence of preference for glycerine media and no unusual characters.

The results of animal inoculations showed that this virus was possessed of only a moderate virulence for rabbits. The animal which received 0.01 mg. intravenously died from advanced disease of the lungs sixty-nine days after inoculation, the lesions being, as would be expected when death had been so long delayed, much more irregular in type and larger than is usual after the inoculation of a strain of high virulence.

The animal inoculated with 10.0 mg. subcutaneously was killed after 131 days, being then in fairly fat condition and showing a moderate degree

of generalised tuberculosis; the glands were unaffected. The two other rabbits, which were given 1 mg. and 0.1 mg. subcutaneously respectively, were affected with lesions of only a slight character, and the lungs were in both cases crepitant.

Two guinea-pigs unfortunately died of other diseases, and the lesions seen at post-mortem were generalised but of a slight character. The 0.1 mg. animal was killed too soon, but the degree of tuberculosis was distinctly less than would be expected if the strain were fully virulent.

The strain, though definitely dysgonic and of bovine type culturally, is thus of distinctly lower virulence than is usually the case with a strain of bovine origin.

It is noticeable that in this case none of the animals receiving antiformined material showed any lesions when killed about a year later.

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
<i>Original inoculations.</i>			
Rabbit ...	Antiformined material from spleen.	Killed 229	Neg.
Guinea-pig.		350	
	Fresh material.	350	
		Died 175	M.T.
<i>Quantitative inoculations with Bedredka culture 529 days later.</i>			
Rabbit ...	10.0 mg. subcut.	Killed 131	M.T.
	1.0	131	S.T.
	0.1	Died 120	S.T.
	0.01 intrav.	69	A.T.
Guinea-pig.	10.0 subcut.	29	E.T.
	1.0	29	E.T.
	0.1	Killed 54	E.T.

CASE VII.

The animal from which this strain was obtained was a stallion, which had been subjected to examination for soundness (including a gallop) nine weeks before destruction. One month after the examination he began to feed less well and to lose condition, and this rapidly increased until about a month later, when respirations were noticed to be thirty-eight per minute, pulse seventy-two, temperature 103.4° F. Stiffness of the neck was not observed. A swelling was noted at the left side of the sheath, from which a little thick caseous pus could be expressed. On the following day the respirations had increased to forty-eight per minute, the pulse to 100, and a little frothy blood occasionally dripped from both nostrils. The animal was destroyed a few days later and a post-mortem examination made immediately. Condition was still fair. The lungs were extensively involved with miliary lesions so close as to appear confluent in the collapsed lung, which was solid, flabby, and increased in weight; the bronchial glands were enlarged and oedematous only, the pleura normal. There was great enlargement of the mesenteric glands with lesions up to 4 cm. diameter, which on section appeared light yellow with deeper yellow or brownish parts, the centre of most being semi-solid, but of a few consisting of a small pocket containing cloudy, whitish liquid,

The spleen appeared normal. One submaxillary gland was involved but all the other carcase glands were free; a tuberculous growth was found in the connective tissue of the sheath; the testicles were normal.

Bacteriological Examination.

Acid-fast bacilli were extremely numerous in the softened material of the mesenteric gland, and fairly numerous in the lung. A small amount of material from the sheath had been received a few days before the other organs, with a report from the veterinary surgeon in attendance that it contained tubercle bacilli. This was confirmed but it was also found to be badly contaminated. Cultures were unsuccessful and the guinea-pig inoculated with an emulsion was chloroformed eight months later, showing small lesions suggestive of tuberculosis in the spleen and liver, but no acid-fast bacilli could be found and cultures were negative.

Strains of this virus were obtained direct and through guinea-pigs inoculated with material from a mesenteric gland and lung, both of which developed tuberculosis though the disease appeared to have advanced slowly.

These strains were less dysgonic than most of the other equine strains, giving a fairly good growth on plain media and on glycerine media after the first generation; cultures appeared noticeably moister and isolated colonies became much larger than in most other dysgonic strains and were definitely stellate and slightly raised.

From the table of animal inoculations it will be seen that the virulence of this strain was distinctly low; 0.01 mg. intravenously took 156 days to kill the animal inoculated, and the lesions then found were quite different from those found after the inoculation of a bovine strain of high virulence and more like the chronic type of disease produced by a human strain. The same remarks apply to the type of disease produced in the animals inoculated subcutaneously

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
<i>Original inoculations.</i>			
Rabbit ...	Emulsion of lung or mesenteric gland.	Killed 153	M.T. (a)
Guinea-pig.		Died 71	M.T.
		73	E.T.
<i>Quantitative inoculations with Besredka culture, 597 days later.</i>			
Rabbit ...	10.0 mg. subcut.	Died 139	M.T. (b)
	1.0	301	S.T. (c)
	0.1	152	M.T. (d)
	0.01 intrav.	152	A.T. (e)
Guinea-pig.	1.0 subcut.	117	M.T. (f)
	0.1	35	E.T. (g)
	10.0	39	E.T. (h)

(a) Lungs, glands, sacculus rotundus, cæcum, and testes all contained lesions of moderate character.

(b) Condition poor, chronic type of disease, extensive lesions of pleura, sacculus rotundus, and cæcum; lungs crepitant, lesions sub-pleural and at edges.

(c) Slight lesions in lungs, kidneys, and testicles.

(d) Lungs showed only small lesions; large lesions in kidneys, glands, and spleen.

(e) Still much normal lung tissue.

(f) Local fibrous lesions. Spleen slightly enlarged and showed numerous lesions up to 2 mm. Glands large and firm, not caseo-necrotic.

(g) Condition good. Spleen lesions up to 1 mm.

(h) Cause of death enteritis. Spleen greyish, $\frac{1}{2}$ -1 mm. centres.

with 10.0 mg. and 0.1 mg. respectively, whilst the rabbit which was given 1.0 mg. died after 301 days, showing only slight lesions in the lungs, kidneys, and testicles. The evidence afforded by the rabbit which received 1.0 c.c. of an emulsion of the original material, extremely rich in bacilli, is in agreement with the quantitative results.

The results can only be interpreted as showing that this virus, though bovine in cultural characters, possessed a virulence markedly lower than that of a typical bovine virus.

CASE VIII.

The only lesion found in this case was a peculiar ulcer in the small intestines. The animal had been in the Royal Veterinary College Infirmary for about two weeks, in poor condition and showing diarrhœa. The ulcer was irregularly oval 7 cm. by 5 cm. and crossed by four distinct transverse ridges, more or less parallel and separated by grooves. It was sharply marked off and had a raised edge, and the colour was pale yellowish-white, contrasting with the surrounding mucous membrane; the serosa was not affected. On section the mucous membrane of the ulcer appeared infiltrated to a varying degree with firm white tissue and below that was much white fibrous tissue.

Bacteriological Examination.

Acid-fast bacilli were found on the surface of the mucous membrane, in its depth, and in the connective tissue under the ridges.

A strain was obtained through a guinea-pig and proved to be of bovine type in the meagreness of its growth and in its high virulence for rabbits and guinea-pigs.

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Material and Dose.</i>		<i>Survival in Days.</i>	<i>Result.</i>
<i>Original inoculations.</i>				
Guinea-pig.	Antiformed ulcer, acid-fasts rare.		Died 15	Local.
<i>Inoculations with Besredka culture 592 days later.</i>				
Rabbit ...	10.0 mg.	subcut.	Killed 48	A.T. (a)
	1.0		48	A.T. (b)
	0.1		Died 84	A.T.
	0.01	intrav.	30	A.T.
Guinea-pig.	10.0	subcut	35	A.T.
	1.0		61	A.T.
	0.1		59	A.T.

(a) and (b) Chloroformed in error, but disease very advanced, the lungs being masses of caseated material.

CASE IX.

This animal was a seven years old cart horse which had worked well until about two months before death, when it was noticed to be losing condition; wasting rapidly progressed and polyuria became a marked symptom. 3 c.c. of tuberculin was injected subcutaneously but no reaction was obtained. Respiration was becoming frequent and difficult and the animal was destroyed.

Post-mortem Examination.

Both lungs were crammed with miliary tubercles. The mesenteric glands were completely altered in shape by tumour-like lesions of the usual type, the

largest being 12 by 7 by 5 cm. In most cases the centre was composed of soft whitish caseo-pus surrounded by a wide zone of greyish tissue with numerous small pockets filled with similar softened material. The spleen was moderately enlarged and studded with rounded projecting lesions up to 8 cm. On section they were composed of firm greyish-white tissue with scattered irregular softened centres.

Bacteriological Examination.

Acid-fast bacilli were present in very large numbers.

A culture was obtained through a guinea-pig and proved to be of dysgonic type, presenting no unusual features.

Rabbits inoculated with this strain survived longer than is usual with a bovine strain and at post-mortem examination showed generalised slight or moderate lesions, in some cases partly calcified. The results obtained in the animals inoculated with the original material were in agreement with these quantitative results and showed that this virus was of distinctly lower virulence than a strain of immediate bovine origin.

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
<i>Original inoculations.</i>			
Rabbit ...	Fresh Spleen	Killed 174	M.T. (a)
		182	S.T. (b)
Guinea-pig.		Died 25	E.T.
		42	M.T.
<i>Quantitative inoculations with Besredka culture. 216 days later.</i>			
Rabbit ...	10.0 mg. subcut.	Died 122	M.T. (c)
	1.0	Killed 40	M.T. (d)
	0.1	Died 124	S.T. (e)
	0.01 intrav.	43	A.T.
Guinea-pig.	10.0 subcut.	46	A.T.
	1.0	50	A.T.
	0.1	33	M.T.

(a) Good condition. Half lung crepitant and lesions widespread.

(b) Fat condition. Cystic local lesion 2 cm. by 1 cm. Nearest gland only affected. Lungs crepitant but riddled with small greyish points. Kidneys negative.

(c) Lungs showed numerous grey translucent lesions up to 2 mm., a few about 3 mm. and partly calcified; major part still crepitant. Kidneys few scars, and few small caseous lesions. Few glands enlarged, but caseation slight. Sacculus rotundus and cæcum affected.

(d) Chloroformed owing to paralysis and retention of urine. Local and slight lesions in lungs.

(e) Lungs crepitant but riddled with grey translucent tubercles up to 2 mm. and few 3-4 mm., caseous and calcified.

CASE X.

The animal was a seventeen years old mule which gradually lost condition for some months before death, lay down a great deal, and showed signs of abdominal pain. Death was stated to be due to peritonitis, following rupture of the intestine.

Post-mortem Examination.

Four large ulcers about 3 cms. by 1.5 cms., of tuberculous type, were present in the small intestine and one much larger, 15 cms. by 9 cms.; the

rupture was believed to have occurred through one of these. The mesenteric glands were reported as enlarged.

Bacteriological Examination.

One acid-fast bacillus only was seen in smears.

A strain was obtained through a guinea-pig and was definitely dysgonic. As it was desired to close this work, no quantitative inoculations were done; one rabbit, however, which was inoculated with an emulsion of the original material in which bacilli were few, died showing advanced generalised tuberculosis after seventy-one days, and it seems probable that this was an ordinary bovine strain.

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
Rabbit ... Guinea-pig.	Antiformed material.	Died 71 15	A.T. E.T.

CASE XI.

The animal was aged twenty years, and had been wasting for about three months. Feeding had been good but emaciation continued and enlargement of the pharyngeal region was observed.

Post-mortem Examination.

The spleen was found to be a little enlarged and distorted by the presence of about fifty lesions of the usual type, mostly 1-2 cm. in diameter but in a few cases up to 4 cm. The pharyngeal glands were much enlarged and composed of whitish tough tissue.

Bacteriological Examination.

Acid-fast bacilli were not rare in the splenic lesions, but none in the pharyngeal glands.

The strain was secured after passage through a guinea-pig and was definitely dysgonic, growing rather less well in the presence of glycerine than without it.

All rabbits inoculated died showing typical generalised lesions, but they survived a little longer than is usual with a bovine strain of average virulence.

ANIMAL INOCULATIONS.

<i>Animal.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
<i>Original inoculations.</i>			
Rabbit ... Guinea-pig.	Fresh spleen and gland.	Died 126 140 180	M.T. A.T. M.T.
<i>Quantitative inoculations with Besredka culture 169 days later.</i>			
Rabbit ...	10.0 mg. subcut.	Died 88	A.T.
	1.0	82	A.T.
	0.1	168	A.T.
	0.01 intrav.	43	A.T.
Guinea-pig.	10.0 subcut.	47	A.T.
	1.0	54	A.T.
	0.1	68	A.T.

CASE XII.

The spleen, mesenteric glands, and a small piece of the lung of this animal were received. The spleen was enlarged and about twice the normal weight owing to the presence of about a dozen tumour-like lesions from 1 cm. to 4 cm. in diameter, a few showing an umbilicated centre; all were firm and otherwise of the usual type. The mesenteric glands were at least four times the normal size and of the usual unsoftened type. The piece of lung was riddled with miliary lesions.

Bacteriological Examination.

Eight splenic lesions were examined and material taken from two, both of which showed numerous acid-fast bacilli; the latter were moderately long, thin, and granular and usually straight. One rabbit and two guinea-pigs were inoculated with fresh material.

Cultures obtained from guinea-pigs were dysgonic and grew rather less well on glycerine than on plain media.

As it was desired to finish off the work, no quantitative tests were made; the strain, however, showed no unusual features, and the result in the only rabbit inoculated was such as to make it probable that the strain possessed the usual bovine virulence.

ANIMAL INOCULATIONS WITH ORIGINAL MATERIAL.

<i>Animal.</i>	<i>Material and Dose.</i>	<i>Survival in Days.</i>	<i>Result.</i>
Rabbit ...	Spleen emulsion.	Died 26	A.T.
Guinea-pig.		40	A.T.
		45	A.T.

CASE XIII.

This animal was reported to have shown symptoms of tuberculosis and reacted to tuberculin (method of use not known). It was accordingly destroyed and the post-mortem examination showed lesions of the lungs, spleen, and mesenteric and submaxillary glands. The spleen only was received, and was of typical character, not much enlarged but showing about twenty lesions of usual type, many with an umbilicated centre. Their size was from 1-3 cm. diameter.

Bacteriological Examination.

Bacilli were very rare, but direct culture was obtained on egg and was of typical bovine type, producing a meagre growth on all media, with and without glycerine, and being highly virulent for rabbits and guinea-pigs.

ANIMAL INOCULATIONS.

Animal.	Weight in grammes.		Material and Dose.	Survival in Days.	Result.
	Initial.	Final.			
Original material.					
Rabbit ...			Fresh spleen.	Died 4	Neg.
Guinea-pig.				Killed 61	A.T.
Rabbit ...			Antiformined spleen.	150	Neg.
				150	
Guinea-pig.				150	
Quantitative inoculations with Besredka culture 164 days later.					
Rabbit ...	2200	1500	10.0 mg. subcut.	Died 44	A.T.
	2200	1700	0.1	76	A.T.
	2200	1800	1.0 intrav.	23	A.T.
	1700	1500	0.01	34	A.T.
Guinea-pig.	595	475	10.0 subcut.	21	E.T. (a)
	610	520	10.0	25	M.T. (b)
	530	435	0.1	49	A.T.
	485	375	0.1	84	A.T. (c)

(a) Spleen, 1/500th of body weight.

(b) Spleen 1/350th of body weight. Liver 1/20th of body weight.

(c) Spleen 1/50th of body weight. Liver 1/7th of body weight.

SUMMARY.

Thirteen cases of tuberculosis in the equines have been investigated and cultures obtained from all, either directly or through the guinea-pig.

All the strains were dysgonic, possessing the cultural characters of bovine tubercle bacilli; in their virulence they differed widely. Eleven were tested quantitatively on rabbits and guinea-pigs, and five of these proved to be of standard bovine virulence, whilst the remainder fell below that standard, four being of definitely low virulence.

Two other viruses (Cases 10 and 12) were not tested in measured doses, but the results obtained with original material give an indication that they were of standard bovine virulence.

SOME EXPERIMENTS ON TUBERCULIN TESTING BY
THE SUBCUTANEOUS AND INTRADERMAL
METHODS,
AND OBSERVATIONS AS TO THEIR COMPARATIVE
VALUES ON YOUNG CATTLE AND DAIRY
COWS, RESPECTIVELY.

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(Continued from page 57.)

THE NEWCASTLE-UPON-TYNE (STANNINGTON) EXPERIMENTS.

The subcutaneous tests were all carried out on the farm premises at Stannington. The testing by the combined ophthalmic and intradermal method of one bull, twelve cows, and four heifers was also done at these premises, the testing of the remaining eighteen cows and one heifer, also by the latter method, being carried out in comfortable cattle sheds within the City of Newcastle-upon-Tyne. The animals were all tag numbered, and after the completion of the testing of each lot they were slaughtered within a slaughterhouse reserved for the purpose within the City, where the post-mortem examinations were made.

The Subcutaneous Tests.

A total of 50 animals, comprising 37 cows and 13 heifers, were tested. The ages of the cows varied from about four to eight years or slightly over. One heifer was $3\frac{1}{2}$ years whilst the remaining twelve varied in age from $8\frac{1}{2}$ to 21 months. The first 40 animals (27 cows and 13 heifers) tested were submitted to the subcutaneous method. The temperatures of the animals were taken during the two days prior to the day of injection of the tuberculin, then at the time of injection and at the 9th, 12th, 15th, and 18th hour afterwards. For the testing of the first 40 animals, including all previous tests, the tuberculin used was obtained direct from the Royal Veterinary College, London, whilst that used for the remaining ten, by the combined ophthalmic and subcutaneous methods, was Pasteur Institute Tuberculin. Also in all previous tests on the last-mentioned animals the same brand of tuberculin was employed except on two occasions, when only the subcutaneous method was applied and Royal Veterinary College tuberculin used. The seat of injection was on the left side of the neck immediately in front of the shoulder region, the ophthalmic test being performed on the same side after examining both eyes for the purpose of detecting any abnormality. To ensure freshness, new supplies of tuberculin were obtained on each occasion immediately prior to the commencement of testing.

The Combined Intradermal and Ophthalmic Tests.

By this method a total of 36 animals, comprising 30 cows, five heifers, and one bull, were tested. The cows varied in age from four to nine years; the bull was two years, one heifer was four months,

one was $9\frac{1}{2}$ months, whilst the remaining three heifers were from two and a half to three years of age.

The tuberculin employed for the testing of these animals was concentrated tuberculin, fresh supplies for the testing of each lot being obtained on each occasion direct from the Field Laboratories, Cambridge University.

The Intradermal Tests.

The site chosen was at about the centre of the side of the neck on the left side. With the use of an ordinary shaving brush, shaving soap, and hot water, an area of about 4-in. by 3-in. was well lathered. Then by means of a safety razor an oblong patch measuring about 3-in. by 2-in. was shaved and afterwards dried with a clean towel.

The animal is secured by means of an ordinary halter on the head, the latter being steadily fixed by an attendant firmly holding the halter shank, which may be more securely held by being fastened to some fitting immediately in front of the animal. The same or another attendant secures a firm hold of the nostrils with the right hand or a pair of bulldogs, the left hand being used to steady the animal by holding one of the horns or otherwise. A 1 c.c. all-metal glass-barrelled syringe with a rather fine needle $\frac{3}{4}$ -in. in length was used for the purpose of injecting the tuberculin, which was carried out in the following manner. With the left forefinger and thumb a fold of the shaven skin is firmly held horizontally. Then, with the syringe in the right hand, the needle is firmly supported by the forefinger and thumb and inserted in an almost horizontal direction into the thickness of the skin, after which, by steadily pressing the piston, the tuberculin is injected. The dose injected is about $1\frac{1}{2}$ drops. When the tuberculin has been successfully injected one finds, on passing the fingers lightly over the seat, a tense pea-sized swelling.

On making a visit 48 hours later, should there be no evidence of any local reaction, a second dose may be given in precisely the same way. Twenty-four hours after the first dose a positive reaction may be detected, rendering it quite unnecessary to repeat the dose. The swelling may persist for several days, although gradually diminishing in extent.

The Local Reaction.

The swelling at the seat of inoculation in a reacting animal is invariably œdematous in character, and in some cases very tense, hot, and painful; in others less so. In some cases the swellings, particularly when very tense, are almost tumour-like in character. In the majority of cases, however, the area has a somewhat inflated appearance, with its outer borders slightly raised above the level of the surrounding or normal skin, and may measure from about 1-in. by $\frac{3}{4}$ -in. up to about 7-in. or 8-in. by 3-in. or 4-in. In the absence of a reaction and on placing the hand flat and firmly against the neck, the remains of the swelling first caused by the injection may be noted, but nothing else. Then, by taking up a fold of skin at the same position between

the finger and thumb, one finds that, by exercising a rotatory movement under pressure by the latter, the animal appears to enjoy the sensation and has a tendency to lean towards one. With a definite reaction the swelling can invariably be seen at a glance in a good light. The extent of the swelling may best be determined by passing the finger tips gently over the side of the neck in various directions, but particularly from various points of the circumference of an imaginary circle towards the centre or seat of inoculation. In almost all cases the reacting animal most definitely resents the approach of one's hand towards the neck region, and even in those cases where the local swelling is very small in extent and not apparently painful pressure between the finger and thumb of the fold of swollen skin is almost always responded to by the animal in a manner that can generally be distinguished from that of a non-reacting animal, notwithstanding the fact that one may experience amongst animals not under any test wide differences in the matter of response to handling.

The Ophthalmic Tests.

Before the instillation of tuberculin the two eyes were compared and examined for the presence of any abnormality, the left one being used for the test. This was carried out immediately after injecting the animal intradermally and before releasing it. The head is slightly turned and steadily held with the left eye placed uppermost. With the syringe in the right hand and with the fingers of the left hand gently pressing the upper lid backwards, about three drops of tuberculin are instilled and then the upper lid is released.

The Reaction.

At various stages during the first 24 hours after instillation a definite reaction may be observed. Whether a doubt exists or not as to the presence of a reaction, it is an advantage, as an aid to diagnosis, to compare the eyes. Sometimes in the reacting animal there may be nothing more than congestion of the conjunctiva, which may or may not be accompanied by a more or less watery discharge passing over the face. There may be considerable swelling of the lids or this may be almost absent. The discharge may contain one or two small collections of scarcely perceptible pus. On the other hand, lumps of pus may be found sticking to the lower lid or collected at the inner canthus. When examined 24 to 48 hours after instillation, the pus may have been carried from the lid or inner canthus on to the face and be found partly or completely dried but still adherent to the hair that has been glued together by the discharge, which may have formed a definite and more or less easily recognised tear-track down the face.

The Post-Mortem Examinations.

After slaughter the carcase and internal organs of each animal were kept separate from others and numbered in accordance with the tag number of the animal concerned. In order to avoid the removal of any evidence of disease, in no case was any stripping, paring, or removal

of fat or other tissues or superficially placed glands permitted until the post-mortem examination had been completed.

Throughout the whole of the examinations in only two cases were these controlled by subsequent microscopic or guinea-pig inoculations. The specimens from these two animals were submitted for that purpose to the Department of Bacteriology, University of Durham College of Medicine, Newcastle-upon-Tyne, and may be briefly referred to as follows :—

No. 64, Cow, 6 years.—Tested twice by the subcutaneous method only, with an interval of twelve months. No reaction on either occasion. Guinea-pigs were inoculated with material from lesions in the right lung and the anterior and posterior mediastinal lymphatic glands. The result was positive in each case.

No. 86, Heifer, 3 years.—Tested by the combined ophthalmic and intra-dermal method. No reaction occurred. This animal had not been submitted to any test previously. The lesion from the bronchial lymph gland was examined microscopically with a negative result ; a doubtful acid-fast organism was found in the lesion from the mesenteric lymph gland.

Two guinea-pigs were inoculated with material from each lesion and on being killed were found to be tuberculous.

Method of Examining the Glands.

When examining the various lymph glands for the presence of tuberculous lesions very thin sections of the entire gland should be made, otherwise, minute lesions, although of macroscopic dimensions, may easily escape detection. For example, if the blade of the cutting instrument be not only too thick but also imperfectly sharpened there is always on making sections a danger of a small lesion, such as a caseous tubercle up to the size of the head of a small collar stud, being left embedded within the thickness of one of the sections made. It appears certain, to the author at least, that failure to take sufficient precaution in this direction or entrusting the work to others, either insufficiently experienced or interested, may result in the tuberculin test on a reacting animal being discredited on many occasions when macroscopic lesions are present but undetected, and, for the same reason, in the test on a non-reacting animal being credited with accuracy which did not exist.

GENERAL OBSERVATIONS.

The Subcutaneous Tests.

Of the tests by the subcutaneous method on 50 animals, the results in 39 (31 positive and eight negative), or 78 per cent., were in agreement with those of the post-mortem examinations. In the remaining eleven, or 22 per cent., the results were all negative but lesions of tuberculosis were found on slaughter in every case. With reference to these non-reactors, it should be noted that one had not been previously tested ; four had been tested once previously, two with negative results, one with a doubtful and one with a positive result. Of the remaining six which had each been tested on two previous occasions, two had given

negative results only, one had given a positive and a negative result, one had given two doubtful results, one had given a doubtful and a positive result, and one had given two positive results. Therefore, whilst four had given positive and two had given doubtful reactions at some previous test, there remained five animals, or 10 per cent. of the total tested, which had given no other than negative results at any time, although found diseased on slaughter.

Amongst the diseased animals which had given negative results to the test immediately before slaughter three may be classed as so-called "ceased reactors." Of these, No. 56 gave two doubtful reactions with an interval of seven months between each, the last being eleven months prior to the time of slaughter; No. 1 gave a doubtful reaction in 1922, a positive reaction in 1923, and three years later a negative result a few days before slaughter; whilst No. 15 gave positive reactions in 1922 and 1923, but three years later gave a definitely negative result just prior to slaughter. One animal (No. 116) was tested in March, 1926, with a negative result, but reacted to the test in March, 1927. Six months later the animal was again tested with a definitely negative result and upon slaughter immediately afterwards tuberculous lesions were found.

The Intradermal Tests.

Thirty-six animals were tested by the combined ophthalmic and intradermal method and in 31, or 86·1 per cent. of the tests, the results (29 positive and two negative) of the intradermal method were in agreement with those of the post-mortem examinations. Of the five animals which had given negative results and were found diseased on slaughter, one had not been previously tested; two had each been tested once previously with negative results; one had been previously tested on two occasions with positive results, whilst one had been tested on four previous occasions with negative results only—three times by the subcutaneous method and once by the combined subcutaneous and ophthalmic method. The other previous tests referred to were by the subcutaneous method only. The animal which in two previous tests by the subcutaneous method had given positive reactions also at the time of testing by the intradermal method gave a positive reaction to the ophthalmic test. Therefore, of the animals found diseased on slaughter, four, or 11·1 per cent. of those tested, had given no other than negative results.

With reference to the combined ophthalmic and intradermal method, it may be noted that in 32, or 88·8 per cent., of the tests, the results were in agreement with those of the post-mortem examinations, and that in the tests by the same method, on animals found diseased on slaughter, in one case, as previously mentioned, the ophthalmic method gave a positive reaction when the result of the intradermal method was negative, whereas in each of three cases the results of the intradermal method was positive in combination with a negative result by the ophthalmic method.

COW No. 5.

April, 1922.	26th	27th	28th		29th		
	Wed.	Thurs.	Friday.		Saturday.		
			3 p.m.	12 p.m.	3 a.m.	6 a.m.	8 a.m.
	101·2	101	100·8	100·6	100·4	101	101·8
April, 1923.	26th	27th	28th		29th		
	Thurs.	Fri.	Saturday.		Sunday.		
	11 a.m.	11 a.m.	12·30p.m.	9·30p.m.	12·30a.m.	3·30 a.m.	6·30a.m.
	101·4	102·2	101·2	102	101·4	100·6	101
	29th	30th		31st			
March, 1926	11 a.m.	2 p.m.	11 p.m.	2 a.m.	5 a.m.	8 a.m.	
	101·4	102	101·6	101·4	101	102·4	
March, 1927	17th		18th			Ophthal- mic Test.	
	Init. Temp						
		9th	12th	15th	18th		
	101	101	101	100·5	101·2	Nil.	
June, 1927.	7th/11th				14th		
	Intrad.		Ophth.		Post-mortem Result.		
	—		—		+		

A Non-reactor to Three Methods of Testing.

Special attention is directed to animal No. 5 (see table.) This animal, an all-red shorthorn cow, was never known to suffer from illness of any description. She was attractive looking, had a beautiful glossy coat, carried a considerable amount of flesh and fat, and, besides being a splendid feeder and good milker, at all times appeared in perfect health and condition.

She was tested three times by the subcutaneous method, namely in April, 1922, April, 1923, and March, 1926, tuberculin from the Royal Veterinary College, London, being used on each occasion. In March, 1927, she was tested by the combined subcutaneous and ophthalmic method, Pasteur Institute tuberculin being used. Three months later she was submitted, by the combined intradermal and ophthalmic method, to a fifth or final test with tuberculin from the Field Laboratories, Cambridge University. The result of the test on every occasion was completely negative. On making a post-mortem

examination within 72 hours after the completion of the final test, lesions of tuberculosis were found in the bronchial, mediastinal, portal, and mesenteric lymphatic glands.

The Results of the Subcutaneous and Intradermal Tests Compared.

It has already been stated that, as disclosed by the post-mortem examinations, whilst the results of the subcutaneous tests carried out on 50 animals were accurate to the extent of 78 per cent, those of the intradermal tests on 36 animals were accurate to the extent of 86·1 per cent.

Of the 50 animals tested by the subcutaneous method 37 were cows varying in age from four to seven years, the remaining 13 being heifers. Of the heifers, six were from eight and a half to twelve months and six were from 15 to 21 months, the remaining one being three and a half years of age.

When the 37 cows were tested the results in 26 (19 positive and seven negative), or 70·27 per cent., were in agreement with the results of the post-mortem examinations, whereas when the 13 heifers were tested the results (twelve positive and one negative) were found to agree with those of the post-mortem examinations in every case.

Of the 36 animals tested by the intradermal method 30 were cows varying in age from four to nine years, the remaining six comprising two heifer calves, one four and the other nine and a half months, one bull two years, and three heifers from two and a half to three years of age.

When the 30 cows were tested the results in 26 (all positive), or 86·6 per cent., were in agreement with the results of the post-mortem examinations, whereas when the remaining six animals (calves, heifers, and bull) were tested, the results in five (three positive and two negative), or 83·3 per cent., were in agreement with the post-mortem examination results.

According to these results, therefore, the intradermal method appears the more reliable for the testing of cows, but for the testing of young cattle the subcutaneous method appears the more reliable of the two.

CONCLUSIONS.

It has already been shown that in herds, particularly dairy cows, producing reactors from time to time, the animals that should be regarded with a certain amount of suspicion are the non-reacting cows, for amongst them are sometimes included diseased animals which neither the subcutaneous nor the intradermal test, alone or combined with the ophthalmic, is capable of detecting. It is not improbable that much of the abuse cast against agriculturists and others from time to time regarding doping or tuberculinising cattle against subsequent successful testing is uncalled for, and perhaps more correctly explained by reference to temporary or permanent "ceased reactors," or animals similar to those which, although tested by the three methods over a period of years, have never been known to react.

With regard to the detection of disease amongst quite young cattle, the following statement in reference to the reliability of the intradermal test appears in the Medical Research Council's Report (page 86): "Hitherto it has been the custom to wait for a positive reaction in the adult animal before disposing of it, owing to unreliable results obtained with the subcutaneous test in young animals under field conditions." . . . "The elimination of the young as well as the mature reactors is now feasible."

Having regard to the fact that the Medical Research Council Committee produced no evidence concerning the reliability or otherwise of the subcutaneous method for the testing of quite young cattle, their statement appears not only unjustified but, unfortunately, somewhat misleading when considered in relation to the facts concerning this method established during the past 20 years. For example, in Rutherford's tests on 39 young animals at least 18 out of the 19 animals found diseased had at some previous time given a reaction to tuberculin and only the one animal remaining had given no other than negative results. This animal had been tested four times and was slaughtered when well over two years old. Again, in the experiments by McFadyean and Sheather, when 50 quite young animals were tested by the ophthalmic, subcutaneous, and intradermal methods for the first time in the case of each animal tested, the results by the subcutaneous method appear to have been 100 per cent. accurate as compared with 98 per cent. accuracy in the results by the intradermal method. Further, these results are confirmed by the writer's experiments, in which the results in young animals were 100 per cent. accurate when tested by the subcutaneous method as compared with 83.3 per cent. accuracy when tested by the intradermal method.

In conclusion it is submitted that :—

I. A positive reaction by either the subcutaneous or intradermal method of testing may be accepted as certain evidence that the animal in question is tuberculous in some degree.

II. A so-called doubtful but definite reaction by the subcutaneous method should, provisionally, be regarded as positive.

III. The ophthalmic test, although generally recognised as a subsidiary test, may occasionally prove the deciding factor when in combination with either of the other two methods.

IV. For testing quite young cattle (from a few months to two or three years of age) for the presence of tuberculosis, either the subcutaneous or the intradermal method may be considered as highly satisfactory.

V. For testing adult cattle, such as dairy cows, the intradermal method is more reliable than the subcutaneous method. The test by either method, however, is not entirely satisfactory because negative results, whether obtained alone or in combination with the ophthalmic method, cannot be relied upon as being accurate, for, on slaughter, tuberculous lesions may be found in one or more of the animals in question.

VI. Further experiments are necessary to ascertain what percentage of adult cattle (diseased animals that fail to react to the methods described) will certainly react to the "associated tests" or a combination of the three methods as recommended by Professor Lignières.

To Mr. William Dotchin, M.R.C.V.S., late of Newcastle-upon-Tyne, and to Mr. C. Nicholson, M.R.C.V.S., Morpeth, Northumberland, I extend my warmest thanks for their valuable assistance in carrying out the subcutaneous and the combined subcutaneous and ophthalmic tests respectively.

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[*Erratum*.—In the previous part of this article, page 55, 13th line from the bottom, the words "in series VI" should read "in the six series."]

THE MERCURIC CHLORIDE TEST FOR CAMEL TRYPANOSOMIASIS.

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(Received for publication, April 18th, 1929.)

I.—INTRODUCTION.

IN an earlier number of this Journal,* a first communication was made on the usefulness of dilute aqueous solutions of mercuric chloride in diagnosing trypanosomiasis in camels. The main conclusion was that the addition of one drop of the serum of an infected camel to one cubic centimetre of a 1-20,000 solution of mercuric chloride was followed by the development of opacity within a few minutes whereas normal camel serum induced no such change. Further evidence was produced to demonstrate the superiority of this test over the formol-gel test which has for the last two years been used throughout the Sudan as a routine method of diagnosis, the points in its favour being that it detects infection in earlier stages, less time is required to obtain a reading, and the readings are more easy to assess. It was shown that the test had never failed to detect the disease in infected camels, but on the question of obtaining consistently negative results in non-infected camels it was considered that sufficient evidence was not available.

The object of this paper is to record the observations made during a further year's study of the test, partly from the aspect of its general reliability—in particular as compared with the formol-gel test—and partly with the object of settling minor outstanding details of technique. In the main these two aspects are very closely related, since in establishing a final technique this must necessarily be one with which consistently negative results are obtained in the case of non-infected camels.

II.—TECHNIQUE.

(1) *Handling of Serum Samples.*

It has been noticed that the method of removing serum from the blood clot is not entirely a matter of indifference. In the earlier publication (*loc. cit.*) it so happened that arrangements were always made for this work to be carried out first in the morning, and in order to have everything ready the serum samples were always removed from the clot the afternoon before and allowed to stand overnight.

Later, owing to pressure of other work, the tests had to be carried out at any hour of the day when time could be spared, and in order to expedite results the routine was tried of taking a blood sample, centrifuging as soon as clotting had occurred, and testing the supernatant serum immediately. From the very first, however, it was observed that this technique did not give satisfactory results; in positive sera nothing abnormal could be noticed, but in the serum of

* Vol. xli, Part 4, pp. 341-353, December, 1928.

TABLE I.
MERCURIC CHLORIDE REACTION OF NORMAL CAMEL SERA SEPARATED BY VARIOUS TECHNIQUES.

Camel No.	Technique 1. HgCl ₂ in 1,000's			Technique 2. HgCl ₂ in 1,000's			Technique 3. HgCl ₂ in 1,000's		
	15	20	25	15	20	25	15	20	25
11	++	—	—	++	—	—	++	—	—
17	++	—	—	++	—	—	++	—	—
19	++	—	—	—	—	—	—	—	—
20	++	—	—	—	—	—	—	—	—
21	++	—	—	?	—	—	?	—	—
22	?	—	—	—	—	—	—	—	—
24	++	—	—	—	—	—	—	—	—
25	++	—	—	—	—	—	—	—	—
26	++	—	—	++	—	—	?	—	—
28	++	—	—	—	—	—	—	—	—
29	++	?	—	—	—	—	—	—	—
30	++	?	—	++	—	—	—	—	—
31	++	?	—	++	—	—	++	?	—
32	++	?	—	++	—	—	++	?	—
34	++	—	—	—	—	—	—	—	—
36	++	?	—	?	—	—	?	—	—
38	++	++	—	++	—	—	++	—	—
39	++	++	—	++	—	—	++	—	—
41	?	?	—	?	—	—	++	—	—
42	++	?	—	?	—	—	++	—	—
43	++	—	—	++	—	—	?	—	—
44	++	—	—	—	—	—	++	—	—
45	++	++	?	—	—	—	—	—	—
46	++	?	—	?	—	—	++	—	—
47	?	—	—	++	—	—	?	—	—
48	++	—	—	—	—	—	++	—	—
49	++	++	?	—	—	—	—	—	—
50	?	++	—	—	—	—	?	—	—
51	++	++	?	++	—	—	?	—	—
52	++	++	—	++	—	—	++	—	—
53	++	++	?	—	—	—	++	—	—
54	++	—	—	—	—	—	++	—	—
55	++	—	—	?	—	—	++	—	—
56	?	?	—	—	—	—	++	—	—
57	++	—	—	++	—	—	++	—	—
59	?	—	—	++	—	—	++	—	—
60	++	?	—	++	—	—	++	—	—
61	++	++	—	++	—	—	++	—	—
62	?	++	—	++	—	—	++	—	—
63	?	?	—	?	—	—	?	—	—
64	++	?	—	?	—	—	++	—	—
65	++	—	—	++	—	—	++	—	—
66	++	++	—	?	—	—	++	—	—
67	++	?	—	++	—	—	++	—	—
68	++	?	—	++	—	—	++	—	—
69	++	—	—	++	—	—	?	—	—
70	?	—	—	++	—	—	++	—	—
72	++	?	—	++	—	—	++	—	—
73	++	?	—	?	?	—	++	++	—
74	++	—	—	?	—	—	++	—	—
75	++	—	—	?	—	—	++	—	—
76	Not done			++	—	—	++	—	—
77	" "			++	—	—	++	—	—
78	" "			++	—	—	++	?	—
81	" "			++	?	—	++	++	—

TABLE I.—*Continued.*

Camel No.	Technique 1. HgCl ₂ in 1,000's			Technique 2. HgCl ₂ in 1,000's			Technique 3. HgCl ₂ in 1,000's		
	15	20	25	15	20	25	15	20	25
82		"	"	+	—	—	+	—	—
83		"	"	+	—	—	+	—	—
84		"	"	+	—	—	+	—	—
85		"	"	+	—	—	+	—	—
86		"	"	?	—	—	+	—	—
87		"	"	—	—	—	—	—	—
88	+	?	—	?	—	—	?	—	—
89	+	—	—	—	—	—	+	—	—
90	+	?	—	+	—	—	+	—	—
91	+	+	—	+	—	—	+	—	—
92	+	—	—	—	—	—	+	—	—
93	+	?	—	+	—	—	+	—	—
94	?	—	—	+	—	—	+	—	—
95	+	Not done	—	+	—	—	—	?	—
96	?	—	—	?	—	—	?	—	—
97	+	Not done	—	+	—	—	+	—	—
98	+	+	—	?	—	—	+	—	—
99	+	—	—	+	—	—	+	—	—
100	+	—	—	—	—	—	?	—	—
102	?	—	—	+	—	—	+	—	—

obviously healthy camels, coming, moreover, from known trypanosome-free areas, a faint bluish or "smoky" effect was usually noticed when the serum was added to 1-15,000 and in rarer instances 1-20,000 solutions of mercuric chloride; the same sera retested after standing overnight usually gave consistently negative results with the 1-15,000 and always with the 1-20,000 solution. It seemed, therefore, that contact with the clot was a matter of some importance, but, since in the casual testing of small numbers of camels no particular correlation could be detected, arrangements were made to test a large batch of healthy young remount camels and to control the results of the test by animal inoculation in every case.

Table I shows the readings of the tests on 76 camels carried out during January and February, 1929. In every case a white rat or gerbil was injected intraperitoneally with one cubic centimetre of blood on the day of the test, and in every case with negative result. The techniques recorded are:—

Technique 1: Blood centrifuged immediately after clotting; supernatant serum tested at once.

Technique 2: Serum removed from clot and allowed to stand (at room temperature) overnight.

Technique 3: Serum allowed to stand in contact with clot overnight.

The numbers 15, 20, and 25 indicate 1-15,000, 1-20,000, etc., aqueous solutions of mercuric chloride.

The signs indicating opacity are interpreted:—

+

 very distinct white opacity.

±

 faint white opacity.

?

 bluish or "smoky" appearance of mixture.

In assessing the records set out in this Table it is immediately evident that Technique No. 2 (serum removed from the clot and stood overnight) is the best ; in only two cases was the reading of the 1-20,000 tube other than water-clear, and even in these cases only a " smoky " appearance and not a white opacity was recorded. Next in order of merit is the technique of allowing the serum to stand in contact with the clot overnight ; with this technique two faint opacities and four " smoky " reactions were recorded with the 1-20,000 dilution but in no case was the 1-25,000 other than water-clear. The records amply confirm the unreliability of the technique of centrifuging a fresh clot and testing the serum immediately.

As a routine method of diagnosis, therefore, choice lies between the techniques numbered 2 and 3 ; if the former be used a 1-20,000 solution of mercuric chloride is suitable, whereas if the latter be chosen it would be necessary to use a 1-25,000 solution ; in any case, infected camels by any technique give very strong reactions at dilutions much higher than 1-25,000. It seems that in consideration of all circumstances, especially those prevailing in the field, the best technique is to allow clotted blood to stand overnight and to test the serum by adding one drop to one cubic centimetre of 1-25,000 mercuric chloride solution.

It is unfortunate that no explanation of these slight variations can be given. The mechanism of the test has been studied (a paper on this subject is in course of preparation), but, as might be expected, the test itself is more delicate than any analytical method designed to work out the chemical changes involved.

(2) *The Mercuric Chloride Solution.*

It was originally thought that with a relatively stable compound such as mercuric chloride the solution when once prepared would be very stable. Such appears not to be the case, and a solution that has been allowed to stand in a glass bottle for several weeks becomes weaker. No special trouble has been taken to explain this, but it is conceivable that with a very dilute solution such as 1-20,000 very little chemical interaction with or adsorption on the glass would effect a considerable reduction in concentration. The difficulty is easily overcome by always using fresh solutions, which for field work could be dispatched at frequent intervals from the laboratory.

III.—GENERAL RELIABILITY.

The earlier publication on this test gave records of 87 camels, in which the presence or absence of infection was definitely known in the case of 86 (24 infected, 62 healthy) while the single remaining case was lost sight of ; it was, however, assumed to be infected and gave positive reactions to the mercuric chloride and formol-gel tests.

During the past year 155 additional camels have been submitted to the test, particulars of which are as follows :—

(a) *Positive reactors.*—Total 39, in 36 of which infection has been proved either by microscopic examination or by animal inoculation.

(b) *Negative reactors*.—Total 116, all of which have been negative by microscopic examination and 76 of which have been confirmed as non-infected by animal inoculation.

It may be further noted that the subsequent history of most of the camels is known, and in no case has trypanosomiasis developed so shortly after a negative reaction to the test as to throw any doubt on its accuracy.

IV.—COMPARISON WITH THE FORMOL-GEL TEST.

The foregoing section constitutes an assessment of the reliability of the test in absolute terms—no discrepancy has been observed in a total of 242 camels tested. It is not without interest, however, to compare it with the formol-gel test, since the latter test has certain points to recommend it, particularly when field conditions in the Sudan are considered. In particular, it is not necessary to have a clean sample of serum—hæmolysis or contamination with sheer dirt do not seem to affect the results. In addition, it has been universally used in this country, field officers are used to performing it, and on the whole its results have been satisfactory; it is not therefore to be discarded except to be replaced by something markedly superior.

(a) *Technique*. Apart from the greater ease with which the mercuric chloride test is carried out, the whole principle of the formol-gel test as at present applied in this country calls for comment. When first introduced in India for the diagnosis of Kala-azar in man it was insisted that the essential positive phenomenon was the development of opacity in formolised serum rather than gelation. Subsequent publications have further insisted on this; indeed it has since been suggested that it be called the “serum-aldehyde” test, in order to eliminate the conception of gelation as an essential phenomenon. As can be appreciated from the work of Knowles,* in this country, it was soon noticed that opacity could not be correlated with trypanosome infection in camels, but that gelation was much more closely associated. Subsequent observations have confirmed this, but nevertheless too many inconsistencies are encountered. Knowles himself pointed out (*loc. cit.*, 1927) that some non-infected camels give “temporary positive” reactions; twelve such cases in a series of 160 are quoted. As a field test this is objectionable since in a large country with few veterinary officers it is not practicable to apply repeated tests. Further, there is every likelihood that at a subsequent test more “temporary positive” reactors will be found even if the original ones have reverted to “negative.” The records of the mercuric chloride test show that hitherto no non-infected camel out of 178 tested has given a “temporary positive” reaction.

(b) *Partial reactions*. Further comment seems to be desirable on the “partial reactions” frequently observed in the formol-gel test, these being readings at which the formolised serum is considerably thickened but not firmly gelled. It can be argued that a “partial”

* (a) *This Journal*, 1924, Vol. xxxvii, p. 37 *et seq.* (b) *Ibid.*, 1927, Vol. xl, p. 59 *et seq.*

reaction is a positive reaction in its early stages or alternatively that it is not; Knowles (*loc. cit.*, 1927) has quoted cases of non-infected camels developing a partial reaction and later returning to negative without treatment. In the course of one's own observations during the last three years both phenomena have been observed.

In fact, in view of two recent publications by Lloyd and Paul,* in India, indicating that the positive phenomenon in human kala-azar is a precipitation of euglobulin, and the confirmation of this fact in the case of camel trypanosomiasis in the Sudan (paper in course of preparation), it seems that gelation is to be regarded as a masking rather than a diagnostic feature; that opacity does not regularly occur in positive formol-gel reactions in camel trypanosomiasis is referable either to the fact that formaldehyde is not a good euglobulin precipitant or that the euglobulin increase in camels infected with trypanosomes is relatively less than in human beings infected with *Leishmania* or both. This objection is eliminated in the case of the mercuric chloride test since in reading the result any degree of opacity in the solution is to be regarded as a positive reaction.

(c) *Incubation period.* The time that normally elapses between infection and the development of a positive reaction to the formol-gel test has always been in doubt. Knowles (*loc. cit.*, 1927) observed three cases in which the periods were 37, 44, and 50 days respectively. This is a very small number and is not sufficient to indicate either that all infected camels develop a positive reaction or that, once developed, it does not subsequently disappear without treatment; in fact Knowles himself records one infected camel in which the latter phenomenon occurred.

Observations on the "incubation period" are necessarily few since subjects are limited to camels experimentally infected in the laboratory, these being the only ones in which the actual date of infection is known. Regarding the mercuric chloride test alone, particulars are available of fourteen camels, most of which received treatment early in the course of the disease and cannot be used for comparison with the formol-gel reaction. Table II shows the time in weeks (tests were performed weekly) for reactions to appear both with 1-20,000 and 1-25,000 solutions according to Technique No. 2—serum removed from clot and allowed to stand overnight.

TABLE II.

"INCUBATION PERIOD" TO MERCURIC CHLORIDE TEST IN FOURTEEN CAMELS.

Mercuric Chloride.	Reaction developed in			
	1 week.	2 weeks.	3 weeks.	4 weeks.
1-20,000 ...	4	10	—	—
1-25,000 ...	1	6	6	1

* *Ind. J. Med. Research*, 1928, Vol. xvi, p. 203 and p. 529.

A comparison has in part been made with this test and the formol-gel test, a summary in the case of eight camels being set out in Table III.

TABLE III.

LENGTH OF TIME FOR REACTION TO DEVELOP TO MERCURIC AND FORMOL-GEL TESTS.

<i>Camel No.</i>	<i>Mercuric chloride.</i>	<i>Formol-gel.</i>	<i>Remarks.</i>
S.S. 88	14 days	Not in 148 days	Condition very bad, treated on 148th day.
S.S. 173	7 days	17 days	Reactions persisted until 103rd day, when camel was treated.
S.S. 218	14 days	48 days	Formol-gel reaction became negative again on 111th day. Camel treated on 132nd day. HgCl_2 positive throughout.
L. 15	14 days	36 days	Positive to formol-gel for two weeks only, "partial" reaction for two weeks more, and from 64th day until 86th day (treated) was negative. HgCl_2 positive throughout.
L. 16	14 days	38 days	Both reactions persisted until treatment on 58th day.
L. 19	14 days	Not in 42 days	Treated on 42nd day, but subsequently died.
L. 20	14 days.	Not in 46 days	Treated on 46th day.
L. 21	14 days	Not in 36 days	Died on 36th day.

Reference to Tables II and III shows that the mercuric chloride test is relatively superior to the formol-gel; all of fourteen experimentally infected camels reacted with 1-25,000 solutions of mercuric chloride and thirteen of them within three weeks, while the records in Table III show in addition that a reaction to the formol-gel test may never develop at all. All the camels recorded in this Table as treated were allowed to remain until they were in a dangerous condition before receiving treatment—in fact some of them were left too long and died, but a persistent formol-gel reaction only developed in two of them.

(d) *Positive reaction in other conditions.* It has always been admitted that certain other conditions are likely to induce the development of a positive formol-gel reaction. Only two such cases have come under notice while studying the mercuric chloride test, particulars of which are as follows :—

(i) *Camel No. L.13.* Suffering from a condition locally known as *guhra* (a chronic cellulitis). The camel was kept under observation for one month only, during which time it gave a persistently positive

formol-gel reaction but was negative to mercuric chloride. Inoculation of white rats was negative.

(ii) *Camel No. L.11.* Generally unthrifty and losing condition. From June 30th, 1928, until November 29th, 1928, its reaction to the mercuric chloride reaction was always negative; the formol-gel reaction, however, was usually positive, occasionally "partial," and at three or four tests negative. The camel was ultimately used in another experiment and died, but the cause of the general emaciation was not determined on post-mortem. White rats inoculated on November 10th, 1928, remained healthy.

It is not desirable to attach great importance to the observations in these two cases; they may not be representative, but at any rate the mercuric chloride reactions were negative in two non-infected camels giving positive reactions to the formol-gel test.

V.—DISAPPEARANCE OF REACTION IN RECOVERED CAMELS.

It was assumed by Knowles (*loc. cit.*, 1927) that the formol-gel reaction would require about the same length of time to disappear in a cured camel as it had taken to develop after infection, but few exact observations were produced in support of this assumption. No attempts have since been made either to support or refute it. Since a positive formol-gel reaction may appear in healthy camels—the so-called "temporary reactors"—and may never appear in infected camels, it would be difficult to assess any evidence brought forward. It is essential, however, to have some fairly accurate knowledge concerning the disappearance of any diagnostic reaction after recovery from a disease; such a reaction might never disappear completely and this fact would have to be considered at later stages in the animal's history.

Up to the present time records have only been collected of eleven camels, three laboratory cases subjected to weekly examination and a batch of eight others which were available for re-examination on two occasions only, 45 and 276 days after treatment. The three camels under personal observation reverted to negative after four, six, and ten weeks respectively, and of the eight other camels three were negative 45 days after cure and the remaining five were negative at the second test on the 276th day.

The evidence on this point is thus neither full nor conclusive; it seems, however, that most recovered camels are likely to give a negative reaction within two or three months of cure, and, in any case, of the eleven tested no positively reacting individual remained nine months after cure.

VI.—OTHER DIAGNOSTIC AGENTS.

It was originally mentioned that mercuric chloride was the best of many compounds employed in working out this class of test. That two such dissimilar compounds as formaldehyde and mercuric chloride should be useful extended the field of experiment indefinitely, but in order to keep the work within practicable limits studies in this Laboratory were confined to metallic salts and a few well known protein

precipitants. Of the metallic salts, the chlorides of nickel, cobalt, and manganese were the most promising, but they failed in not drawing a distinct line between weak positives and strong negatives. Salts of gold, copper (cupric), iron (ferric), cadmium, lithium, barium, strontium, calcium, yttrium, and aluminium appeared to draw no line at all. Of the organic compounds tried picric acid was the best, and could almost be used to replace mercuric chloride in case of necessity. No healthy camel was found to react with a 1-10,000 solution whereas infected camels ranged up to 1-100,000 or more before arriving at the stage where pure water gave a reaction. The objection to picric acid is merely that the opacity produced is always very faint. Sulphosalicylic acid behaves similarly to picric acid, but the precipitates produced are even fainter.

It is not desirable to discuss these other compounds at length; they may be useful for other conditions, but for single compound tests they have been discarded in favour of mercuric chloride in the diagnosis of camel trypanosomiasis.

VII.—SUMMARY.

1. A further year's study of the mercuric chloride test has confirmed the view that it is superior in every way to the formol-gel test for the diagnosis of trypanosomiasis in camels.

2. The best technique for use in routine tests in the field is to allow blood to clot, and test the serum the following day by adding one drop to one cubic centimetre of a fairly freshly made 1-25,000 aqueous solution of mercuric chloride. A positive reaction consists in the appearance of opacity within a few minutes.

3. In a total of 242 camels submitted to the test all infected camels have given a positive reaction and all non-infected have given a negative reaction. This constitutes a great improvement on the formol-gel test, in which appreciable numbers of healthy camels give positive reactions and some infected camels fail to do so.

4. Two cases are quoted in which a positive formol-gel reaction was associated with other chronic conditions, but in neither of these was there a positive reaction to the mercuric chloride test. It is, however, difficult to distinguish between conclusions under this section and those under section 3.

5. The difficulty of "partial reactions," so often observed in the formol-gel test, does not arise.

6. The period elapsing between infection and positive reaction is two or three weeks, as contrasted with the formol-gel test in which it is always longer (possibly averaging six weeks), and in which a positive reaction may never develop. The short "incubation period" is of great importance in detecting the more acute cases.

7. As far as a limited number of retests can be accepted as representative, a positive reaction seems to disappear within about two months of cure (with Naganol).

In conclusion, I have to thank Mr. P. A. C. Kenny for carrying out many of the observations during my absences from Khartoum.

THE FILTERABLE VIRUS OF TUBERCULOSIS.

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IN 1910, Fontès¹, working at Rio de Janeiro, claimed to have produced in guinea-pigs, inoculated with filtrates of tuberculous material passed through Berkefeld candles, a slow hypertrophy of the lymphatic glands, with the appearance of typical tubercle bacilli in the gland tissue. As other workers were unable to confirm these claims, the matter was, apparently, allowed to lapse into obscurity.

In 1922 the subject was re-opened by Vaudremer², who, during the course of other experiments, definitely proved the existence of a filterable virus of tuberculosis, thereby confirming the original findings of Fontès. Arloing and Dufaurt³ have, however, pointed out that acid-fast bacilli are not invariably accompanied by a filterable virus. The importance of Vaudremer's work was immediately recognised, numerous workers investigated the question, and the existence of a filterable virus of tuberculosis was established beyond doubt.

The inoculation of filtrates into guinea-pigs is usually followed by a variable degree of hypertrophy of the lymphatic glands. The virulence of the filterable virus is slight, but, according to Arloing and Thevenot⁴, varies in a similar manner to different strains of the tubercle bacillus.

Its virulence, however, can occasionally be exalted by passage through guinea-pigs, as has been shown by Valtis, Nègre, Boquet, and Mlle. Centoncin⁵, and more recently by Saenz⁶.

Mlle. Togounoff⁷ has failed to exalt the virulence by this method.

According to Valtis⁸, the majority of guinea-pigs inoculated with filterable virus die between the third and fourth months. In our experience the majority of such guinea-pigs remain apparently healthy.

Valtis and his co-workers⁵ found acid-fast bacilli in the omentum and associated lymph glands of guinea-pigs two days after the intra-peritoneal inoculation of filtrate of young cultures grown on Sauton's synthetic medium.

Valtis⁸ states that acid-fast bacilli are most easily found in the lymphatic glands during the two months following inoculation, and that after this period they gradually tend to disappear.

According to De Sanctis Monaldi⁹, acid-fast bacilli cannot be found after the third month in the gland smears of guinea-pigs inoculated with filtrates. Most of our positive results have been obtained during the fourth month, earlier examinations of guinea-pigs from the same groups having given negative results. In view, however, of the rarity of bacilli in smears and the prolonged searching necessary to find them, this may be coincidence.

It is interesting to note that Verge and Lesbouyries¹⁰ also delay the examination of smears until after the third month, while Mlle. Togounoff¹¹ examines them at intervals varying from the second to the sixth month after inoculation.

In the majority of our negative cases bacilli morphologically resembling the tubercle bacilli were found, but the degree of acid-fastness shown was insufficient to warrant their acceptance as typical tubercle bacilli.

In view of the statement of Cooper and Petroff¹² that they found acid-fast bacilli in the lymphatic glands of 33 per cent. of their apparently normal guinea-pigs, we have made a prolonged examination of gland smears of ten of our stock guinea-pigs, but neither acid-fast bacilli nor acid-fast granules were found. De Sanctis Monaldi⁹ has examined gland smears of ninety-two healthy guinea-pigs with negative results.

In order to eliminate from our work the possible influence of the personal factor, those organisms only were accepted as tubercle bacilli which satisfied an independent member of the Laboratory staff, usually the Director, Dr. W. H. Andrews.

Many investigations have been made recently on this subject, but chiefly with tuberculous material and cultures of human origin.

Lesbouyries and Verge¹³ have demonstrated the presence of filterable virus in tuberculous material of canine origin. De Potter¹⁴ has shown that young cultures of avian tubercle bacilli also contain the filterable virus.

In so far as we have ascertained, Rossi¹⁵ appears to be the only investigator who has worked with material of bovine origin. He has proved the existence of the filterable virus in the milk of a cow affected with tuberculous mastitis in addition to generalised tuberculosis.

The literature on the filterable virus of tuberculosis has been fully reviewed in recent publications by Valtis⁸ and by Calmette¹⁶.

The experiments here described have been carried out with the milk and blood of cows in various stages of infection with tuberculosis.

Technique.

The same technique was employed in each milk experiment. The milk was autolysed at 37° C., for 48 hours, diluted to ten times its volume with 0.85 per cent. salt solution, passed through filter paper, and then through a Chamberland L₂ candle under a negative pressure of 15-ins. Hg.

The filtration, carried out at room temperature (14°-16° C.), never occupied more than ten minutes.

The reliability of the filter was controlled by adding 10 c.c. of an 18 hour broth culture of *B. avisepticus* to the liquid before filtration, and 5 c.c. of the filtrate was sown in Martin's broth and incubated at 37° C. for one week.

Before incubation the blood was diluted to ten times its volume with 3 per cent. solution of sodium citrate in distilled water.

A negative pressure of 20-ins. Hg. was employed and filtration was allowed to proceed for 15 minutes. The technique was similar in other respects to that described for milk.

In each experiment the filtrate was inoculated into six guinea-pigs; three were given 10 c.c. intraperitoneally, and three 5 c.c. subcutaneously.

These guinea-pigs were isolated in a special house in order to eliminate the risk of accidental infection with tuberculosis. The control guinea-pigs were kept in a separate house.

New instruments, slides, and filters were used throughout the experiments.

The examination of inoculated guinea-pigs was confined to the bronchial glands. These were removed with the usual precautions, cut into small pieces and crushed between slides, and smears were made from the gland juice.

In some of our earlier attempts the glands were crushed, suspended in normal saline and centrifuged, and smears made from the deposit. This method was not satisfactory.

Smears, stained in the usual way with warm carbol fuchsin, were decolourised with 33 per cent. nitric acid for 20 seconds, followed by 90 per cent. alcohol for 30 seconds, and counter-stained with methylene blue.

MILK.

Experiment No. I.

Cow No. 469, in good condition, affected with tuberculosis in near hind quarter of udder. The milk from the affected quarter was thick, yellow, and flocculent in appearance. Tubercle bacilli were found on microscopical examination. A small quantity of milk was collected in a sterile flask and two control guinea-pigs were inoculated with it, one intraperitoneally and one subcutaneously; they died of generalised tuberculosis in twenty-one and fifty days respectively. The remainder of the milk was incubated and filtered according to the described technique, and the filtrate was inoculated into six guinea-pigs.

Twenty-seven days later a guinea-pig inoculated intraperitoneally was killed and smears were made from the bronchial glands. A prolonged search failed to reveal any acid-fast bacilli.

A second guinea-pig, inoculated intraperitoneally, was killed on the fifty-third day, but examination again failed to reveal acid-fast bacilli.

The third guinea-pig, inoculated intraperitoneally, was killed on the one-hundred-and-sixteenth day, and after prolonged searching of smears a group of acid-fast bacilli, indistinguishable morphologically from tubercle bacilli, was found. While these bacilli were unquestionably acid-fast, the degree of acid-fastness was slightly less than that of normal tubercle bacilli.

Experiment No. II.

Cow 494, in good condition, affected with tuberculosis of near fore-quarter of udder. The milk of the affected quarter was altered to a thick, yellow, flocculent fluid. Tubercle bacilli were not found in it on microscopical examination.

The two control guinea-pigs inoculated with it, one intraperitoneally and one subcutaneously, died of generalised tuberculosis in forty-nine and twenty-two days respectively. The remainder of the milk was inoculated and filtered in the usual way, and the filtrate was inoculated into six guinea-pigs.

The examination of gland smears of a guinea-pig inoculated subcutaneously and killed on the fifty-seventh day failed to reveal acid-fast bacilli.

A second guinea-pig, inoculated subcutaneously, was killed on the one-hundred-and-eleventh day, and smears of the bronchial glands showed a

small group of definitely acid-fast bacilli. The majority of these were short and thick in appearance, but several were long, slender, slightly curved, and indistinguishable from tubercle bacilli.

Experiment No. III.

Cow No. 497, affected with advanced pulmonary tuberculosis. There were no clinical signs of disease in the udder, and the milk was normal in appearance. Microscopical examination of a mixed sample from the four quarters failed to reveal any acid-fast bacilli. Two control guinea-pigs were inoculated, one intraperitoneally and one subcutaneously. The guinea-pig inoculated subcutaneously died on the one-hundred-and-sixteenth day, but no visible lesions of tuberculosis were present. The guinea-pig inoculated intraperitoneally remained apparently healthy. The milk was incubated and filtered according to the usual technique and the filtrate was inoculated into six guinea-pigs. One of the guinea-pigs inoculated intraperitoneally was killed on the one-hundred-and-tenth day. The bronchial glands were two to three times the normal size. After prolonged examination of gland smears two small groups of short, thick, acid-fast bacilli were found. One bacillus longer than the others was indistinguishable from a tubercle bacillus.

Experiment No. IV.

Milk No. 5938, received from Dr. Hare, Royal Veterinary College. The history of the cow was unknown. The milk was altered in appearance to a whey-like liquid containing many flocculi. Microscopical examination showed it to be heavily infected with tubercle bacilli.

Two control guinea-pigs inoculated with it died of generalised tuberculosis in thirty-five and seventy-seven days respectively. A sample of the milk was incubated and filtered according to the routine technique, and inoculated into six guinea-pigs. Two of these died in twenty-nine and forty-two days respectively, without showing lesions of tuberculosis. Gland smears were not examined. A guinea-pig inoculated subcutaneously was killed on the one-hundred-and-twenty-sixth day, and, after prolonged examination of smears, one acid-fast bacillus, indistinguishable from a tubercle bacillus, was found.

A second guinea-pig inoculated intraperitoneally was killed on the one-hundred-and-thirty-second day, and a small group of acid-fast bacilli, indistinguishable from tubercle bacilli, was found in the gland smears.

Experiment No. V.

Milk (J.I.) received from Mr. D. Johnson, M.R.C.V.S. The cow was affected with tuberculous mastitis, in addition to advanced generalised tuberculosis. The milk was normal in appearance and examination of the centrifuge sediment failed to reveal any acid-fast bacilli.

Two control guinea-pigs inoculated with it, one subcutaneously and one intraperitoneally, died of generalised tuberculosis in fifty-four and sixty days respectively.

A sample of the milk was incubated and filtered according to the routine technique, and the filtrate inoculated into six guinea-pigs.

A guinea-pig inoculated subcutaneously was killed on the one-hundred-and-twenty-fifth day, and several single acid-fast bacilli, indistinguishable from tubercle bacilli, were found in gland smears.

Experiment No. VI.

Milk (J.2) received from Mr. D. Johnson, M.R.C.V.S. This sample was taken from a cow in good condition affected with clinical tuberculosis in the

near-fore and off-hind quarters of the udder. Microscopical examination showed the milk to be heavily infected with tubercle bacilli.

Two control guinea-pigs inoculated with it, one subcutaneously and one intraperitoneally, died of generalised tuberculosis on the ninety-eighth and forty-fourth day respectively.

A sample of milk was incubated and filtered according to the routine technique, and the filtrate was inoculated into six guinea-pigs.

Four of these guinea-pigs died on the thirty-fourth, forty-second, forty-ninth, and fifty-first days respectively after inoculation, but no acid-fast bacilli were found in the gland smears.

A guinea-pig inoculated intraperitoneally was killed on the one-hundred-and-seventh day and several small groups of acid-fast bacilli, indistinguishable from tubercle bacilli, were found in the gland smears.

BLOOD.

Experiment No. I.

Cow No. 469. This was the same cow as used in the milk Experiment No. I. Blood was drawn from the jugular vein into a solution of sodium citrate in distilled water to make a dilution of 1 : 10. Two control guinea-pigs were inoculated, one intraperitoneally and one subcutaneously, with the citrated blood. The guinea-pig inoculated intraperitoneally died on the twenty-sixth day. No visible lesions of tuberculosis were found on post-mortem examination. The bronchial glands were slightly enlarged, but prolonged examination of smears failed to reveal any acid-fast bacilli. The guinea-pig inoculated subcutaneously remained apparently healthy.

The citrated blood was incubated and filtered according to the routine technique, and the filtrate was inoculated into six guinea-pigs.

One of these guinea-pigs, inoculated intraperitoneally, died on the thirty-second day. It was very emaciated and showed slight enlargement of the bronchial glands, but examination of smears failed to reveal acid-fast bacilli. A guinea-pig inoculated subcutaneously was killed on the one-hundred-and-twelfth day. The bronchial glands were about four times the normal size. A considerable number of single acid-fast bacilli were observed in the gland smears, the majority being short and thick, but a few long slender forms were indistinguishable from tubercle bacilli.

Experiment No. II.

Cow No. 491, affected with advanced pulmonary tuberculosis. Blood was drawn from the jugular vein into a solution of sodium citrate in distilled water to make a dilution of 1 : 10. Two control guinea-pigs were inoculated, one intraperitoneally and one subcutaneously, with the citrated blood. The guinea-pig inoculated subcutaneously died on the thirty-third day. No visible lesions of tuberculosis were found on post-mortem examination. Gland smears were not examined. The guinea-pig inoculated intraperitoneally remained apparently healthy.

The citrated blood was incubated and filtered according to the routine technique, and the filtrate inoculated into six guinea-pigs.

One of the guinea-pigs inoculated subcutaneously was killed on the one-hundred-and-fourth day. Examination of gland smears gave negative results.

A second guinea-pig, inoculated intraperitoneally, was killed on the one-hundred-and-tenth day. A small group of acid-fast bacilli, indistinguishable from tubercle bacilli, was found in the gland smears.

Experiment No. III.

Cow No. 639, affected with pulmonary tuberculosis. Blood was drawn from the jugular vein into a solution of sodium citrate in distilled water to make a dilution of 1 in 10. Two control guinea-pigs were inoculated, one intraperitoneally and one subcutaneously, with the citrated blood. The guinea-pig inoculated intraperitoneally died on the thirty-fourth day without visible lesions of tuberculosis. Gland smears were not examined. The guinea-pig inoculated subcutaneously remained apparently healthy.

The citrated blood was incubated and filtered as usual, and the filtrate inoculated into six guinea-pigs.

A guinea-pig inoculated subcutaneously was killed on the one-hundred-and-fifth day. The bronchial glands were about twice the normal size, but examination of smears showed no bacilli sufficiently characteristic to justify acceptance, although at least half-a-dozen of very suspicious forms were observed.

A second guinea-pig, inoculated intraperitoneally, was killed on the one-hundred-and-eleventh day. The glands were about three times the normal size. A brief examination of smears resulted in finding eight to ten acid-fast bacilli indistinguishable from tubercle bacilli.

SUMMARY.

1.—Six specimens of milk and three of blood obtained from cows affected with tuberculosis have been tested by guinea-pig inoculation for the presence of the filterable virus of tuberculosis.

2.—Acid-fast bacilli indistinguishable from tubercle bacilli have been found in gland smears of guinea-pigs inoculated with filtrate of each specimen of milk and blood.

3.—In one instance acid-fast bacilli indistinguishable from tubercle bacilli were found in gland smears of a guinea-pig inoculated with filtrate of milk from the apparently healthy udder of a cow affected with pulmonary tuberculosis.

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We take this opportunity of expressing our thanks to M. Valtis, Pasteur Institute, Paris, for advice on the technique; and to Dr. Hare and Mr. D. Johnson, M.R.C.V.S., for specimens of milk.

A RENAL CARCINOMA IN A BROWN BEAR.*

By C. BRUCE PERRY, M.D., M.R.C.P.

*University Centre of Cardiac Research, General Hospital,
Bristol.*

THE following case seems worthy of record as showing the occurrence of an adeno-carcinoma of the kidney in a Brown Bear (*Ursus arctos*).

The subject had lived for thirty-four years in captivity in the Clifton Zoological Gardens, and was nearly full grown when it arrived there. It was killed, as for the last twelve months it had shown increasing signs of senility. The body was sent to the Anatomical Department of the University of Bristol for dissection. The heart, lungs, and kidneys were kept for my examination, which unfortunately was not made until the other organs had been destroyed. However, no abnormalities were noticed in the dissection other than the tumour in the right kidney. The heart, lungs, and left kidney were normal. The right kidney showed a large nodular tumour situated in the upper half of the organ. On section the tumour is pale in colour, with large areas of hæmorrhage and necrosis, and has entirely replaced the upper lobules of the kidney. It is surrounded by a dense capsule of fibrous tissue, but in places it has broken through this, and the fresh invasion has again been encapsuled with fibrous tissue.

Microscopically the tumour is composed of polygonal cells, which in some places show an attempt at acinous formation. There is very little connective tissue present, and the vessels are poor and ill-supported. The cells contain a fairly abundant cytoplasm, which shows no vacuolation, and the nuclei vary greatly in size and staining properties, while some show mitotic figures. The tumour is surrounded by fully formed fibrous tissue, within which there are in some places marginal collections of lymphocytes. The neighbouring kidney parenchyma shows pressure atrophy.

* I should like to express my thanks to Professor E. Fawcett for kindly letting me examine the specimen, and for permission to report it; to Dr. Richard Clarke for information as to the bear's life history; and to Dr. A. L. Taylor for his kind help and advice.

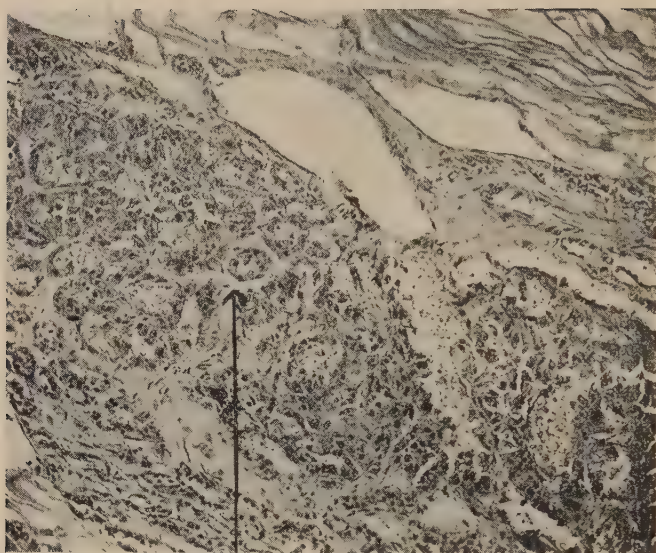


FIG. 1.

Section of the kidney showing attempt at acinous formation (*arrow*).

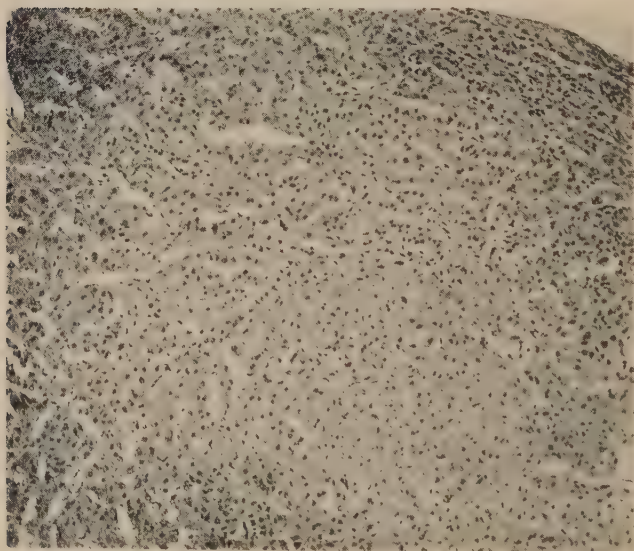


FIG. 2.

Shows the usual arrangement of the cells in the tumour.

THE INCIDENCE OF TUBERCULOSIS IN A HERD IN NORTHERN RHODESIA.

By HARVEY S. PURCHASE, B.Sc., M.R.C.V.S.

THE herd under consideration is one of the oldest established in the Fort Jameson district of Northern Rhodesia. It is composed chiefly of Angoni cows crossed with Hereford and Shorthorn bulls. The incidence of tuberculosis in the herd, as revealed by the subcutaneous tuberculin test, is of interest when compared with the average degree of infection in Great Britain and other countries where cattle are more generally housed and stall-fed.

The tests were carried out during the period October to December, 1928, using tuberculin supplied by the Royal Veterinary College, London. The cows and bulls were injected with 3·3·5 c.c., and young stock with 2·2·5 c.c.

The temperatures of the animals were taken prior to injection, and those over 103° F. were not tested. A number of cattle had a consistently high temperature on several occasions, spread over two to four weeks. These were finally tested. The temperatures of two such animals are given :—

<i>Name.</i>	<i>Pre-inoculation.</i>	<i>12 hours.</i>	<i>15 hours.</i>	<i>18 hours.</i>	<i>Result.</i>
Chisoti II ...	104·3 and 104·2	103·4	104·2	104·8	Non-reactor
Makoza I ...	103·1 and 103·0	104·9	106·1	106·5	Reactor

In Rhodesia where certain protozoan diseases are widespread, and are liable to cause irregularities of temperatures, the efficacy of the subcutaneous test is obviously prejudiced.

The stock is divided into three herds, known respectively as the breeding, oxen, and young stock herds. The breeding herd consists of cows and in-calf heifers. The young calves are allowed to suckle during the day and are kept apart from their mothers throughout the night. In the morning the cows are milked before allowing the calves to feed.

The oxen, which are one year old and upwards, are trained and used for various kinds of work. These are housed and grazed separately. The young stock consists of heifers and bullocks, aged from six to eleven months. The heifers are kept in this herd until mature for breeding, and the young bullocks are similarly retained until ready to be broken in.

These three herds are brought every night into a Cattle Kraal, which is an unroofed and spacious enclosure bounded by walls twelve feet high. Each herd is separated from the others by a six-feet brick wall. The young calves are housed at night in a roofed building until weaned, when they are drafted into the "young stock" herd.

Tuberculosis had not previously been observed on the farm at post-mortem examinations. Special search for it was not carried out, however, until the disease manifested itself on autopsy of a number of cows. On discovering this, it was decided to test the whole herd, and in July, 1928, the tuberculin was despatched. Mr. F. E. Jones, M.R.C.V.S., Government Veterinary Officer, Fort Jameson, kindly undertook the supervision of the testing.

	<i>No. tested.</i>	<i>Reacted.</i>	<i>Per cent.</i>
Total of cattle	222	45	20
Breeding herd	80	10	13
Oxen	68	16	24
Young Stock	74	19	26

These results do not include calves under six months.

Sixty of the "young stock" were tested at the same time as their mothers. Only two of the nineteen (20 per cent.) reacting "young stock" were progeny of reactor mothers. Seven of the reacting cows had reared calves which did not react.

REMARKS.

(1) The lowest percentage of reactors was in the "breeding herd" (13 per cent.). This is of interest when compared with the incidence of tuberculosis in milking herds in Great Britain, where most authorities place it at about thirty to thirty-five per cent.

(2) The highest percentage of reactors (26 per cent.) was in the "young stock." As the disease is of recent introduction this would, in my opinion, appear to indicate that the focus of infection is in the "breeding herd."

(3) The high percentage of reactors among the oxen is difficult to explain, as they are rarely worked to any great age. The percentage is probably an index of the degree of infection in the "young stock" during the previous two to three years.

(4) In view of the ranching conditions under which cattle are kept in Rhodesia, the total percentage of reactors (20 per cent.) in the herd was surprisingly high, and particularly so when compared with the average herd infection in Great Britain, generally estimated at from ten to fifteen per cent. where the conditions are more favourable for the dissemination of infection.

(5) The chief mode of infection for the "young stock" is puzzling. With such a low percentage of reacting cows the udder can hardly be incriminated.

(6) The rapid ingress into the herd made by tuberculosis suggests as a feasible explanation that the native cattle are more susceptible to the relatively newly introduced disease. This has been found the case with certain races of human beings.

(7) A test not depending on a thermal reaction is probably the best for use in warm climates. The subcutaneous test may be interfered with by an intercurrent infection, and especially by a fluctuating temperature due to one of the protozoan blood diseases.

Subsequent to the testing of the herds eleven post-mortems have been carried out on animals killed or dead, from various causes. The tuberculin test was in each case verified by finding distinct macroscopic lesions in those cattle that had reacted, but no lesions could be detected in non-reactors. (The biological test by using guinea-pigs could not be carried out).

The reactors have been segregated and the whole stock will soon be retested.

TARTAR EMETIC SOLUTION IN THE TREATMENT OF NAGANA IN ZULULAND.

By F. G. CAWSTON, M.D. (Cantab.).

H. E. HORNBY, F.R.C.V.S., has reported the results of some post-mortem investigations which seemed to show that the large and repeated doses of tartar emetic which had been recommended in the treatment of cattle suffering from Nagana were heroic and unnecessarily large.

Ll. E. W. Bevan,* M.R.C.V.S., has submitted evidence to show that these doses are not commonly associated with undesirable toxic effects, and he associates toxæmia with the unreliable preparations of the drug.

Amongst the impurities which have been isolated from tartar emetic as sold for oral administration, traces of lead are perhaps the most common and most to be feared. It is only since practitioners have recognised that what is sold for oral use is not necessarily fit for intravenous injection that disagreeable effects of the intravenous administration of tartar emetic have become infrequent.

When first employed, intravenous antimony was expected to cause a rise in temperature. This might have been partly due to the large quantities of distilled water that were employed in preparing the solution for intravenous work; for an injection sometimes resulted in the passing of a considerable quantity of urine. Even where there could be no question of the purity of the water, the system might conceivably react unfavourably to the injection of much distilled water into the venous circulation, especially where the liver substance was impaired by the presence of blood parasites. Many practitioners prefer to use boiled tap-water in preference to distilled water, but saline is undoubtedly to be preferred.

The introduction of tablets containing pure tartar emetic combined with sodium chloride has rendered intravenous injections of tartar emetic a much more simple and safe method of treatment. Farmers in

* A method of inoculating cattle against Trypanosomiasis. By Ll. E. W. Bevan, M.R.C.V.S., Trans. of Roy. Soc. Trop. Med. & Hyg. Vol. xxii., No. 2, August, 1928.

Zululand have sometimes used these tablets, dissolved in small quantities of boiling tap-water, shortly before the injection has been given, for Nagana in their cattle. An additional advantage of this saline solution of the drug is that the accidental escape of a few drops outside the vein or into its wall causes less local reaction than is common without the addition of saline.

The tablets may be obtained from any reliable druggist and are far cheaper than other preparations of antimony that are used in the treatment of Nagana. When, for any reason, farmers are unable to obtain them readily, sodium chloride might well be added to the tartar emetic powder supplied by the Department of Agriculture, when the solution is being prepared for use.

There is no doubt that fresh solutions of tartar emetic are to be preferred; the growth of fungus in stale solutions indicates that they may cause undesirable toxic effects, and I am glad to have Mr. Hornby's statement that he did not understand why I had so strongly advocated fresh solutions, for it had always been his practice in Rhodesia to employ fresh solutions of the drug.

NOTE ON MERCURIC CHLORIDE IN THE DIAGNOSIS OF TRYPANOSOMIASIS IN CATTLE.

By H. E. HORNBY, O.B.E., F.R.C.V.S.,
Tanganyika Territory.

FOR the information of those readers who are interested in trypanosomiasis, but who are not workers on the subject, it may be advisable to point out that although the interesting article by Bennett and Kenny on "Mercuric Chloride as a Diagnostic Agent for Trypanosomiasis in Camels" (Vol. xli, Part 4), is valuable to the degree indicated by the title, it must not be construed as implying that the test has a wider application.

As the authors state, the test is probably dependent on a physical reaction between the proteins of serum and the salt, resembling that resulting in a formol-gel. Therefore, as it is well known that the formol-gel test is useless in bovine practice, it is hardly to be expected that the new test will be otherwise.

I compared the reactions given by sera from eight healthy oxen with those given by sera from two cattle infected with *T. congolense*. The result showed no appreciable difference; seven of the control sera gave reactions identical with the test ones, namely, a distinct opacity when one drop of serum was added to 1 c.c. of a 1-40,000 solution of Mercuric Chloride.

The authors are to be congratulated on their improvement of a valuable test for Surra in Camels, and I wish they could tell us of something as simple and effective for the diagnosis of Nagana in cattle.

CLUB FORMATIONS SURROUNDING DEAD TUBERCLE BACILLI AS REACTIONS ON THE PART OF THE TISSUES OF THE HOST.

By K. MEYER and E. MAYER.

THE occurrence in cultures of branching forms of tubercle bacilli and of bacilli showing thickened ends has long been known, and this morphological character has been held to indicate a relationship between the tubercle bacillus and the ray fungi. Babes and Levaditi have described tuberculosis lesions containing bacilli arranged in a radiating fashion. The literature also contains a few references to the production of radial arrangements and club-formation in lesions caused by tubercle bacilli.

In connection with the authors' attempts to immunise animals, rabbits were inoculated with dead human tubercle bacilli and in three cases there were lesions in the lungs in which the ray fungus arrangement of the bacilli was found.

Two animals had received 4 injections of 5 c.c. of an emulsion of dead tubercle bacilli intravenously at intervals of five days. The first was killed one month, and the second eight days, after the last injection. The third animal had been given two injections at an interval of nineteen days, and was killed five days after the second. The emulsion was prepared by grinding up as finely as possible dried tubercle bacilli in 500 times the quantity of salt solution. Microscopic examination showed that clumps of bacilli were still present. In two cases the bacilli were killed by being subjected to steam at 100° C. for half an hour, while in the third they were subjected to autoclaving for half an hour at 110° C. Guinea-pig inoculations proved that the bacilli had been killed.

Paraffin sections were prepared from the lung lesions after fixation of the tissues in formalin, and these were stained by the methods of Gram, Ziehl-Neelsen, and Koch-Ehrlich, and also for histology.

In places there could be found Gram-positive acid-fast bacilli which were for the most part included within giant cells, and collections of acid-fast granules and homogeneous acid-fast masses of a size similar to the clumps of bacilli. Scattered acid-fast bacilli were also discoverable.

Elsewhere there could be found clumps of gram-positive acid-fast bacilli surrounded by a ring of Gram-negative, eosin-stained, radiating, club-like structures. Finally, in one case structures composed entirely of eosin-stained clubs were found, in which no bacteria could be detected. There could be no doubt that all the Gram-positive acid-fast masses were in really composed of the bacilli injected.

The authors suggest that the appearances presented by the different groups of organisms found in the sections represent a succession of stages. The earliest stage is the cluster or clump of acid-fast Gram-positive bacteria. This is followed by the formation of a gelatinous zone. Later this zone becomes scalloped and finally divided into club-like structures. This suggestion regarding the change occurring in the bacillary clumps is to be the subject of further investigation. The authors feel, however, that their observations throw light not only upon the tuberculous lesion under discussion but also upon the lesions of actinomycosis.

The views generally held with regard to the club-shaped structures which are found surrounding colonies of actinomyces and clumps of tubercle bacilli in lesions, are that the radiating filaments undergo a gelatinous degeneration, or that the club-like terminal swellings are produced by a secretion from the filaments, which results in the formation of a capsule-like structure.

In the authors' experiments the appearances presented in the lesions cannot be explained on either of these grounds since dead bacilli were used. There could have been neither mycelial outgrowth nor formation of a capsule-like structure by the organisms.

The authors cannot find any support for the view that the substance of which the clubs are composed is derived from the bacilli. They suggest that it is at first in the nature of an exudate, as in its earliest form, as interpreted by them, it occurs as a zone which is not differentiated into clubs.

The process of club-formation may occur either around clumps of bacilli included within giant cells or free, and they figure such a clump within a giant cell around which deeply eosin-stained clubs stand out against the paler eosin tint of the cytoplasm of the cell. They also figure and describe a clump of bacilli in which an undifferentiated zone was recognisable along one side, while on the other club-formation was taking place. The outer margin of the undifferentiated portion showed slight crenation. As club formation proceeds the cytoplasm of the giant cell and the coagulation zone disappear, and the clubs stand in close relationship to numerous leucocytes. Giant-cells which contain clumps of bacilli are gradually invaded by leucocytes and destroyed, and the process of club-formation appears to run parallel with this.

It is a separate question as to why the destruction of the gelatinous zone should lead to the formation of clubs arranged in a radial fashion, but it is suggested that it is due to the direction of the "diffusion streams" which pass out from the bacillary clumps.

When the authors' investigations had been completed there appeared, in 1926, a paper by Levaditi and Dimanesco-Nicolan, which afforded support to their views. Levaditi and his co-worker shewed that club formation could be induced by the intravenous injection of tellurium in oily suspensions. They held that the club formations were the result of reaction on the part of the host tissues and that they were composed of an albumen-mineral compound.

The club-formations which the present authors found in the lesions produced by dead tubercle bacilli form a kind of connecting link between those produced by living organisms such as actinomyces and those produced by the tellurium injections.

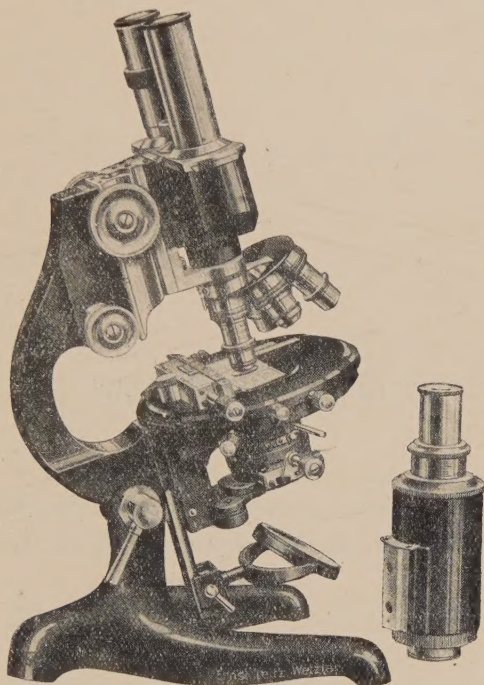
Reference is made to other cases in which club-formation results from the introduction of foreign substances, bacterial or not, into the tissues.

The authors attempted to get an insight into the factors concerned in the process by varying their doses in quantity and number, and in the length of the intervals between consecutive doses, but they failed to get any constant results. (*Zeitschr. f. Hyg. u. Infektions Krankh.*, Vol. 108, No. 1, Oct., 1927, pp. 38-53.)

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